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EFFECT OF FINE GRINDING UPON FLOUR

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It is a fact well known to millers that grinding flour too fine injures its baking quality. The writers have found no report of an adequate investigation of the mode of action of mere attrition upon flour. There are, however, some publications which bear more or less on the question. Thus J. Richardson (1914) reported that flour containing a large quantity of granulates which pass through a No. 21 silk bolting cloth tend to lump, and the baking quality is affected. From this one might conclude either that the finer particles of a flour are inherently of poorer baking quality or that this portion of the flour has been made so by being reduced to great fineness. LeClerc, Wessling, Bailey, and Gordon (1919) pursued the question further. By means of bolting cloths they separated a number of flours into fractions of particles of different sizes. They found the fractions with particles of the larger sizes to contain more ash and somewhat more protein than those consisting of the finer particles. The gluten and the bread obtained from the different separates differed in quality. The separates with particles of intermediate size produced better bread than either the coarser or finer ones. One of the conclusions these investigators reached is that "the very fine granulate is by far the poorest part of the flour, as regards both the quantity and quality of the gluten and as regards the quality of the bread produced from it."

No one seems to have determined upon what the poorer quality of the finer separates depends. It might be the result of the accumulation in them of an undue proportion of offal. This possibility is supported by the observation of LeClerc and his collaborators that the ash content of the finest separate was greater than that of the original flour. It is, therefore, possible that its inferiority may have been due, at any rate in part, to concentration of offal in it, for it is well known to millers that to conduct the milling process in such manner as to produce many fine particles, especially in the early stages of

the reduction, increases the difficulty of removing the offal. On the other hand, it may be that the gluten of the finest separate is inherently of poor quality. Or it may be that such gluten, as it occurs in the wheat berry, is not of poor quality but has been made so by the mechanical treatment it received whereby it was reduced to such a high degree of fineness. If fine grinding reduces baking strength, then this fact would favor the idea that the gluten quality of the finest portions was injured in the milling process. However, it is very probable that the gluten found in the different portions of the same wheat berry is not everywhere of the same quality, as different mill streams contain gluten of different quality (Hendel and Bailey, 1924). It is possible that in the milling process the portions of the wheat berry which contain the poorer gluten find their way in greater proportion into the finest portion of the flour than into the coarser.

At any rate, so far as the writers have been able to learn, no adequate explanation of the well-known fact that over-fine grinding reduces baking quality has been offered. The study the writers have made of the effect of the fine grinding upon the properties of wheat starch (1924) led them to interest themselves in this problem. They had found that grinding starch changes it so that it disperses in cold water and that such dyes as Congo red easily stain the granules. Huss (1922) has also found that mechanically injured starch granules stain with Congo red and swell in cold water, although apparently he did not recognize that they also disperse. He found, moreover, that in normal flour a few starch granules are stained by Congo red, and he surmised that these are the ones that have been injured mechanically. He suggested that in overground flours a greater or lesser portion of the starch granules is injured, though he did not pursue the subject further or suggest how injury to the starch granules brings about damage to the flour. It seemed, therefore, worth while to analyze the effect of fine grinding upon flour, having in mind its effect upon starch.

If fine grinding of flour injures starch in flour in the same way that starch is injured when ground by itself, then overgrinding should increase the cold water extract of flour, as some of the starch will then disperse in water. That such is the case is stated by Wanklyn and Cooper (1881). If their observations be sound, then two results of fine grinding are conceivable, either of which may influence baking quality.

The first of these is that in dough made from overground flour there must be more than the normal amount of soluble carbohydrate. This might affect the texture of the dough.

The second possibility is that such soluble carbohydrate, if it be present in overground flour, may be more readily attacked by diastase than uninjured starch. If this be true, then it is possible that overgrinding increases the diastatic action of a flour upon its own starch, which, because it is injured, is less resistant to diastase. Rumsey (1922) and Collatz (1922) have shown that one of the most important factors in flour strength is diastatic action. If diastase converts injured starch grains more readily than uninjured ones, then the initial rate of fermentation of doughs from overground flours should be increased as compared with the same flour before overgrinding.

Finally, there is the possibility that fine grinding injures the gluten as well as the starch.

The writers, therefore, decided to investigate the following questions:

I. Does fine grinding increase the cold-water extract of flour? If it does, to what substance or substances is the increase due?

II. Is the rate of diastatic conversion of starch in flour increased by overgrinding; and is, in consequence, the fermentation rate of overground flour different from that of the same flour before overgrinding?

III. Does fine grinding affect the quantity or quality of the gluten that can be washed from flour?

IV. To what extent does overgrinding injure baking strength?

It was hoped that the answers to these questions would add to our knowledge of the factors that contribute to the baking strength of flour.

Flours were overground in two ways. Most of them were ground in a small porcelain laboratory ball mill with flints. A single sample, a hard northern spring family brand patent, was overground for the writers by L. H. Bailey, of the United States Bureau of Chemistry, on the smooth rolls of an experimental laboratory mill. The writers desire to take this occasion to express to Mr. Bailey their sincere appreciation of this and other courtesies. Mr. Bailey passed the flour through a bolting cloth of given mesh. The portion that did not pass through the cloth was put through the smooth rolls of the mill[1] and again bolted. The process was repeated until all the flour except a negligible amount of fibrous material had passed through the cloth. This trace of tailings was then added to the bolted flour. The original flour passed through a standard bolting cloth of 109 mesh. From it were prepared four overground flours passing respectively through

[1] For a description of the mill see: The Government's Experimental Flour Mill. The Operative Miller, August, 1919, p. 259.

standard bolting cloth of 150 (No. 15XX), 166 (No. 18XX), 173 (No. 20XX), and 178 (No. 21XX) mesh.

All the flour samples studied in the present investigation differed from those examined by LeClerc, Wessling, Bailey, and Gordon (1919) in that they contained the whole of patent flours reduced to a given fineness. The samples studied by LeClerc, Wessling, Bailey, and Gordon were separations of the granulates of different sizes existing in a normal flour, which presumably had not been overground. Each fraction contained, therefore, only a portion of the original flours, whereas the samples studied by the writers in each case contained the whole of the flour.

In addition to the flour overground by Mr. Bailey, a short patent Turkey red flour manufactured locally upon a midget marvel mill was ground in the pebble mill 36 hours. It was then distinctly whiter and smoother to the touch than the unground flour.

A larger portion of the same flour was next ground for 53 hours. The longer time of grinding was necessary because examination with the microscope after 36 hours showed that the flour was not so completely shattered as the first sample ground 36 hours. Evidently attrition takes place more slowly when the mill carries a larger charge.

Other flours, not so strong as the Turkey red flour, were similarly overground. Most of these were California flours made from blends of Pacific wheats, sometimes with a small amount of Eastern wheat.

Under the miscroscope the Turkey red flours overground in the pebble mill exhibited an appearance quite different from that of the normal flour. Before overgrinding, the starch granules appear to be clumped together as if by a matrix of gluten, which holds them together and which stains deeply with Congo red, although the granules themselves within the matrix do not stain. However, not infrequently stray starch granules separated from the matrix take the stain. Very few starch granules swell in cold water. In the overground flour, on the contrary, the matrix had been broken up, the clumps of starch granules scattered and a very large proportion of them could be stained with Congo red and swelled in cold water. For comparison, a commercial pastry flour was examined. It contained fewer clumps of starch imbedded in the gluten matrix than the normal Turkey red flour, but the starch granules, though scattered, did not seem injured. At least there was not a much larger proportion, if any, of granules that swelled and took up Congo red in cold water than in the untreated Turkey red flour.

The original untreated hard spring patent flour obtained from Mr. Bailey, when treated with an aqueous solution of Congo red, showed

no swollen stained granules. After overgrinding to pass through a No. 15XX bolting cloth, it showed a very few; the No. 18XX, a few more, i.e., several to a field; the No. 20XX, many quite swollen granules; and in the finest, No. 21XX, the majority were so far injured that the greater number took up the dye more or less. This finest flour, under the microscope, appeared not unlike the Turkey red flour which had been ground 36 hours in the pebble mill as above described.

I. Cold Water Extract of Normal and Overground Flour

Cold water extract was determined by the method of the Association of Official Agricultural Chemists (1919) for the untreated Turkey red flour, for the same flour after grinding 36 and 53 hours, respectively, and for the hard spring flours overground by Mr. Bailey. The results are collected in Table I.

TABLE I

EXAMINATION OF COLD WATER EXTRACTS OF NORMAL AND OVERGROUND FLOURS

	Flour extracted	Ash in extracted solids	Reducing substance as dextrose after inversion	Nitrogen (Kjeldahl) in extracted solids
	Per cent	Per cent	Per cent	Per cent
	6.22	....	....	....
	5.87	....	....	....
Cold water extract of Turkey red flour	6.36	3.60	3.88	4.23
before grinding	6.54	5.34	3.62	4.10
	9.08	....	....	....
Same flour ground 36 hours	9.33	....	....	....
	14.85	2.35	7.92	2.05
Same flour ground 53 hours	14.40	1.87	7.75	1.96
	5.27	....	....	....
Hard spring flour before overgrinding	5.19	....	....	....
	5.39	....	....	....
Same flour ground to No. 15 mesh	5.30	....	....	....
	5.26	....	....	....
Some flour ground to No. 18 mesh	5.31	....	....	....
	6.23	....	....	....
Same flour ground to No. 20 mesh	6.07	....	....	....
	6.28	....	....	....
Some flour ground to No. 21 mesh	6.06	....	....	....

Examination of the table shows that the cold water extract increases progressively with the overgrinding. If this be true, the table indicates that the hard spring flour overground by Mr. Bailey was not nearly so badly disintegrated as the Turkey red flour overground in the pebble mill, for its cold water extract was increased but slightly as compared with the flour ground in the pebble mill.

The table also shows that the percentages of ash and nitrogen in the cold water extracts are decreased by overgrinding. The absolute amounts are practically unchanged by the overgrinding process. The increase in extract is, therefore, due mainly to substances that after acid hydrolysis reduce alkaline copper solution—presumably starch. These substances were determined by the official Munson-

Walker method (1919). Their increase is sufficient to account for the increase of water-soluble substances produced by overgrinding. This accords with the statements of Wanklyn and Cooper.

II. Diastatic Activity of Overground Flour and Fermentation Rate

To determine the diastatic activity of the normal and overground flours, the method of Rumsey (1922) was used, the cuprous oxide obtained being estimated by titration with thiosulphate (A. O. A. C. Methods of Analysis, Pt. VII) (1919). The normal Turkey red flour after one hour's digestion at 27° C. showed a reducing power equivalent to 1.25% maltose; the same flour ground for 53 hours, a reducing power equivalent to 5.24% maltose. Overgrinding has, therefore, increased the diastatic conversion in the flour more than four times. It is altogether unlikely that this is due to activation or stimulation of the diastase. It is most probably due to the fact that overgrinding renders the starch more accessible to the diastase. This possibly takes place in two ways. The fine grinding causes much of the starch to disperse in water, as is evidenced by the increase in the cold water extract. This is probably the main factor—a direct effect of attrition on starch. The other way, by which the starch is unlocked, is a more purely mechanical one. In normal flour much of the starch is imbedded in clumps of gluten. Very probably such starch granules are less accessible to diastase than granules that are free. Fine grinding, by shattering the clumps of gluten, sets many granules free and renders them more easily open to attack by diastase.

The rate of fermentation of overground flour was studied by the method of Osterhout for the determination of respiration as adapted by Bailey and Johnson (1924) to dough fermentation. In mixing the doughs 3 grams yeast, 0.5 gram of salt, and 15 to 20 cubic centimeters of water at 28°C. were used for 25 grams of flour. No sugar was added. Four flours were tested: the normal Turkey red flour; the Turkey red flour ground 53 hours; the normal flour from Mr. Bailey; and the same flour ground to pass a No. 21XX bolting cloth. Table II gives the results. The columns bearing the legend "Time required to change pH," etc., give the time required for the carbon dioxide lost from the dough to change the hydrogen-ion concentration of the buffer solution in the absorption tube from pH 7.8 to pH 7.0. These are, therefore, direct measures of the rate of loss of carbon dioxide from the dough. The shorter the time, the more rapid the loss. The rate of loss is the resultant of two factors, namely, the rate of formation of carbon dioxide and the power of the dough to retain enmeshed the gas formed. (For a detailed discussion of the method the reader

is referred to the original paper by Bailey and Johnson.) The first measurements were not made until the dough had been in the thermostat 30 minutes. At the end of the experiments the doughs from the flours supplied by Mr. Bailey, as well as those from the normal Turkey red flour, were well risen and light and porous in consistency, whereas the dough from the overground Turkey red flour was scarcely enlarged in volume and heavy and soggy to the touch.

TABLE II

RATE OF CARBON DIOXIDE LOSS FROM FERMENTING DOUGHS OF NORMAL AND OVERGROUND FLOURS

Time after immersion in thermostat	Time required to change pH of buffer solution in absorption tube from pH 7.8 to pH 7.0			
	Normal Turkey red flour	Overground Turkey red flour	Normal hard spring flour	Overground hard spring flour
Minutes	Seconds	Seconds	Seconds	Seconds
30	522	161	600	433
40	...	157	...	...
45	310	...	...	...
50	...	152	480	220
60	169	154	...	168
65	...	...	250	...
70	154	153	...	171
80	133	146	...	152
90	127	129	159	149

Examination of Table II shows that in both the overground flours the loss of carbon dioxide early in the fermentation is much more rapid than for the corresponding normal flours. How much of this is due to more rapid gas formation resulting from the more rapid inversion of the starch in the overground flour, how much from inability to retain the gas, these experiments alone do not permit one to say. In the overground Turkey red flour both factors probably play a rôle, as the rate of loss does not change very strikingly throughout the experiment. In the overground hard spring flour, increased early gas formation is almost certainly the major factor both because the rate of loss changes in the same general manner as in the normal flours and because, as will be shown later, this flour has not been injured materially by overgrinding. Its baking strength has not deteriorated.

III. Effect of Overgrinding Upon the Quantity and Quality of Gluten that Can Be Washed from Flour

Gluten was determined for the series of more or less overground and normal flours described above. The washing was done with tap water rather than with the buffer solution recommended by Dill and Alsberg (1924), because it was intended to determine ash in the washed gluten. The results of these analyses are given in Table III.

While some of the flour samples have already been described, a description of the different flours reported upon in Table III is here given to facilitate study of the table.

A is the normal Turkey red flour before overgrinding in the pebble mill.

B is a small portion of A ground 36 hours in the pebble mill. Its appearance under the miscroscope has been described above.

C is a different larger portion of A, the Turkey red flour, which was ground 36 hours in the pebble mill. It was not as completely shattered as B, tho it was ground for the same number of hours, because, as above stated, the rate of grinding is influenced by the size of the charge in the mill.

D is C ground for 17 hours, so that it had been ground in a pebble mill 53 hours in all.

E is the hard spring flour furnished by Mr. Bailey.

F is E ground by Mr. Bailey, as above described, until it passed nearly completely through No. 15XX bolting cloth.

G is E ground like F to pass through a No. 18XX bolting cloth

H is E ground like F to pass through a No. 20XX bolting cloth.

J is E ground like F to pass through a No. 21XX bolting cloth.

The washing of gluten from B, C, and D presented some peculiarities. To obtain a homogeneous dough from them required longer kneading than in the case of the unground flour. The dough is crumbly and cheesy in texture. Moreover, it seemed to require more water to wet the flour and secure a uniform dough. It was more difficult to wash the gluten, and the wash water was opalescent from dispersed starch instead of being milky white from suspended starch granules. In the case of B and of D the gluten finally obtained was soft, soapy, and sticky—vastly poorer in quality than that of the unground flour. In the case of C the gluten in the earlier stages of washing seemed of similar quality but, as the washing neared completion, the quality improved and in the end was fairly good.

The washing of gluten from all the other samples presented nothing unusual.

Attempts were made to wash gluten from other overground flours. The results are not included in the table, because in these cases the flours were apparently so badly injured by the grinding that no gluten at all could be washed from them.

When the glutens of B and D were heated in an oven at 100° C. to determine moisture, they expanded hardly at all. The contrast with the normal was quite striking.

Inspection of Table III shows that the badly overground flours, B and D, and also to a lesser extent C, yield less wet gluten than the same flours before overgrinding; that, however, the glutens are more

TABLE III

GLUTEN CONTENT AND COMPOSITION FROM NORMAL AND OVERGROUND FLOURS

	Wet gluten	Dry gluten	Protein content of dry gluten	Total ash of dry gluten average	Water absorption per gram of protein	Gluten quality
	Per cent	Per cent	Per cent	Per cent	Grams	
A. Turkey red flour before overgrinding.........	51.0	14.65	73.5	....	3.32	good
	52.4	15.00	72.7	....	3.37	"
	51.1	14.60	74.0	....	3.31	"
	48.9	13.98	74.2 }	.72	3.30	"
	50.6	14.75	74.2 }	....	3.20	"
B. Do. ground in pebble mill for 36 hours........	41.5	13.3	82.4	....	2.08	poor
	42.9	13.89	82.4	....	2.28	"
	43.1	13.60	81.6 }	1.39*	2.15	"
	41.0	13.15	81.6 }	....	2.55	"
C. Do. second grinding of A in pebble mill for 36 hours, larger charge......................	43.5	13.18	84.2	....	2.69	fairly good
	46.7	14.00	79.5	....	2.81	" "
	44.0	13.10	87.7	....	2.66	" "
	45.5	13.59	83.5	....	2.77	" "
D. Portion of C ground for 17 more hours, total 53 hours	40.6	12.91	88.6	....	2.39	poor
	42.6	13.51	85.0	....	2.50	"
E. Hard spring before grinding..................	34.45	11.49	79.6	.45	2.46	good
	33.79	11.30	80.3	.438	2.43	"
F. Do. on smooth rolls to pass No. 15 standard bolting cloth	35.21	12.70	80.0	....	2.16	"
	35.82	12.20	80.0	....	2.37	"
	34.80	11.95	80.0	....	2.34	"
	31.90	11.05	85.5	....	2.17	"
G. Do. on smooth rolls to pass No. 18 standard bolting cloth	36.66	12.13	78.5	....	2.52	"
	36.85	12.19	79.0	....	2.51	"
H. Do. on smooth rolls to pass No. 20 standard bolting cloth	36.90	12.44	79.0	....	2.43	
	37.70	12.65	79.0	....	2.45	"
I. Do. on smooth rolls to pass No. 21 standard bolting cloth	37.70	12.51	82.3	.50	2.40	"
	38.81	12.91	80.0	.52	2.46	"

*Acid insoluble ash of this dry gluten, 0.578 per cent.

purely protein, for they contain less water and more nitrogenous matter. The absolute amounts of protein obtained in the form of gluten from the same amount of flour are not very different, but their water capacity is very materially reduced as compared with the normal flour.

Flours F, G, H, and J, which were overground between smooth rolls, show little injury from this treatment so far as the gluten analyses indicate. Such effect as is evident is in the direction of improvement. The trend of the data, so far as the limited number of analyses permit one to judge, is in the direction of higher wet gluten yields and higher protein content of the glutens with about the same absorption per gram of protein. Apparently the shattering of the flour has not gone far enough to produce decided results even though in the case of J, as described above, the microscope picture shows the effects of overgrinding.

In all the more or less overground flours there seems to be a tendency for the gluten washed out to be freer from non-protein matter. This is natural enough. On the other hand, there seems to be a tendency for the absolute amount of flour protein recovered in the gluten to increase slightly, provided the overgrinding has not been so extreme as to make gluten washing very difficult or impossible. It is strange that comminution of the gluten masses in the flour does not favor protein losses. As grinding divides the gluten and, therefore, exposes a larger surface to the wash water, one would expect the contrary. It may be that in the doughing process the greater surface exposed favors water absorption and mechanical coalescence of gluten particles. This phenomenon will be studied further, for it may have an important bearing on the methods for the determination of the gliadin-glutenin ratio.

Another effect of overgrinding, if carried to extremes, as in the pebble mill ground fluor, is to reduce the water absorbed per gram of gluten protein and to injure its quality as evidenced by feel and appearance of the washed gluten. It is worth while to inquire whether this is a direct effect of the grinding upon the gluten or whether it is indirect. It is conceivable that the presence of swollen and dispersed starch may affect the texture of the gluten that would otherwise be quite normal. As the ground starch swells in the doughing process, there might be a sort of competition for the water between the swelling starch granules and the gluten. This might result in an imperfect hydration of the gluten during the doughing period, which is possibly not later compensated for during the washing. In harmony with this thought is the observation above recorded that the overground flour required more water to dough and yielded a shorter, rather

crumbly dough. It is, however, also possible that the effect is due to the bulkiness of the swollen starch which perhaps prevents by its physical presence the coalescence of the gluten particles during the "development" of the gluten during doughing. Or possibly the injured starch granules are dispersed out of the gluten matrix, leaving vacuoles. Perhaps these collapse so that the gluten assumes a denser, less open texture. This does not seem very probable, as overgrinding rather thoroughly scatters the gluten masses and sets most of the starch granules free. Moreover, the longer the flour is ground, the more the starch is freed from its gluten matrix, but the worse the gluten. In any event the result of overgrinding may well be the production of a faulty physical texture of the gluten which is not subsequently remedied in the washing process.

Still another possibility is that the presence of much dispersed starch during the "development" of the gluten has the same effect as the presence of much sugar in making the dough "slack" or "runny." One would, however, expect the effect to disappear in the subsequent washing. Finally, the action upon the gluten may be indirect through the effect of the overgrinding on the electrolyte content of the washed gluten. It may well be that from a very finely ground flour some electrolytes are more completely extracted in washing, others more completely absorbed, so that the gluten finally obtained has an abnormal ash. The presence of electrolytes, it is well known, has an important effect on gluten quality.

Obviously, to test the various possibilities it would be desirable to separate gluten from normal and from overground flour by some purely mechanical means which would not produce any change, chemical or physical. Gluten thus isolated could then be ground and studied. Unfortunately no such method is known. The writers have found an approach to such a method by which it is possible to concentrate the gluten in a portion of a lot of flour so that the gluten content is increased by about 50 per cent.

This separation is accomplished by suspending a flour in a mixture of carbon tetrachloride and ordinary gasoline in such proportions that the mixture has a density of exactly 1.464. In this liquid much of the starch with some gluten settles to the bottom, while much gluten with some starch rises to the surface. The middle zone of the liquid contains material which neither rises to the surface nor settles to the bottom. The lighter gluten-enriched portion may be skimmed off and the adherent tetrachloride and gasoline allowed to evaporate spontaneously. In this manner from the Turkey red flour with a nitrogen content of 2.11%, a concentrate was obtained with a nitrogen

content of 3.19%. To make this separation successfully it is necessary to adjust the density of the liquid exactly. Even slight deviations make the separation impossible.[2]

The writers washed gluten from flours treated with carbon tetrachloride-gasoline to learn whether or not such treatment injures the gluten. It was found that the quality is more or less injured, especially if the flotation liquid, which is a good fat solvent, is not drained off thoroughly. In that event the gluten quality is much more seriously impaired than if the flotation liquid is thoroughly removed. Presumably this is because of the difficulty of wetting the gluten due to the changed distribution of the lipoid material contained in it. This observation is interesting in connection with the observations of Working (1924) and Dill (1925). It also gives some hints concerning one of the possible ways in which the presence of germ which contains much lipoid injures flour. It is also suggestive in regard to the manner of action of shortenings.

While the injury to the gluten is considerable when the carbon-tetra-chloride-gasoline mixture is not well removed, if it be removed, the injury to the washed gluten as indicated by its physical properties and water content is not nearly so great. Indeed, it is much less than the injury above recorded as resulting from severe overgrinding of flour. It seems worth while, therefore, to study the effect of overgrinding upon a flour which has been enriched in gluten by flotation. Accordingly a portion of the Turkey red flour was enriched in gluten, as above stated, until it had a nitrogen content of 3.19%. The gluten was washed from a portion without further treatment. Another portion was ground in the pebble mill 20 hours. The effect was exactly the same as for the flour before it was enriched in gluten by flotation. Gluten washing was difficult and the gluten secured was of poor quality with a reduced water capacity per gram of protein. The results then support the idea that severe overgrinding injures gluten directly. They are not conclusive because the gluten-enriched flour still contained much starch; in fact, while its gluten content had been raised about 50%, it contained only from 6 to 7% less non-gluten material than normal flour. Unfortunately it was not found possible to prepare flour fractions with less starch by purely mechanical means.

Having failed to eliminate the starch as a factor because of failure to remove most of the starch from the flour, the problem was next attacked from the opposite direction. Normal flour was enriched

[2] This method was devised by C. L. Alsberg and B. Warner in the course of an investigation, as yet unpublished, in which it was necessary to prepare starch from such seeds as peas without the use of such reagents as KOH, commonly used in purification.

with ground starch and the effect upon gluten washed from it determined.

From a portion of the normal Turkey red flour, starch was prepared by the method used by Rask and Alsberg (1924). The starch was separated into two portions, one being ground in the mill for 118 hours. Admixtures of the flour with the starches were made so that one set contained 5 grams of whole starch to 25 grams of flour; the other 5 grams of ground starch. The mixture was thoroughly sifted four times. Twenty-five grams of each mixture were washed in duplicate to determine gluten. The samples containing the ground starch were noticeably rather more difficult to dough and their behavior in the beginning of gluten washing was very similar to that of the overground flour. That is, the mass was sticky, slimy, and difficult to manipulate. However, as soon as the starch was washed out, the remaining gluten seemed to be of as good quality as that washed from the normal flour, or rather from the sample which had had the normal starch added to it. The conclusion seems warranted that under the conditions of the experiment ground starch added to flour does not affect the quality of the gluten obtained by washing. The analytical data of the experiments follows.

TABLE IV

ADMIXTURES OF GROUND AND UNGROUND STARCH TO TURKEY RED FLOUR GLUTEN DETERMINATIONS (OFFICIAL METHOD)

Wet gluten	Dry gluten	Water in gluten	Imbibition	Protein content gluten, N X 5.7	Absorption per gram gluten protein	Ash in gluten
Per cent	Per cent	Per cent	Per cent	Grams	Grams	Per cent
Unground Starch Added						
39.30	11.56	27.74	70.6	9.26	2.94	0.67
42.10	12.29	29.81	70.7	9.10	3.20	0.60
Ground Starch Added						
41.35	12.60	28.75	69.7	8.55	3.26	0.73
40.01	12.12	27.89	69.7	9.15	2.98	0.73

Effect of Overgrinding a Flour Upon the Ash Content of the Gluten Washed from It

It is possible that grinding a flour in a porcelain mill with flints increases the ash content sufficiently with abraded porcelain and flint to affect materially its properties. To gain information on this point, samples of Turkey red flour before and after grinding were ashed. The ash content rose from 0.389% to 0.419%, an amount so small as to be negligible in the connection herein under consideration.

The effect of the grinding upon the ash content of the gluten washed from the ground flour was, however, different. In the normal gluten from the unground flour the ash content was 0.716% with 0.238% of acid insoluble ash, while in the ground flour gluten the

content rose to 1.44% and 0.578%, respectively. Grinding the flour just about doubled the total ash of the gluten washed from it and increased the acid-insoluble ash somewhat more, i.e., a trifle less than two and one-half times.

A California family flour ground 24 hours in the pebble mill was examined in the same way. For the normal flour the ash content of the gluten was 0.517%, while the ground flour gluten had an ash content of 1.59%; the acid insoluble ash was 0.60%.

It is obvious that severely overground flour yields a gluten with very much greater ash content than normal flour and that the increase is not due in the main to contamination in the mill. If it were, the increase of ash would be entirely acid-insoluble. It is difficult to assign a reason for the increased ash content of gluten from overground flour. It may be that the gluten in overground flour adsorbs electrolytes more completely because, being disintegrated, it presents a greater adsorbing surface, or it may be that the dispersion of the starch in the overground flour liberates some of the mineral matter in the starch and thus makes more electrolytes available for adsorption.

To determine whether the dispersed starch contributes to the ash of gluten from overground flour, the glutens obtained in the above-described experiments in which the gluten was washed from flour to which in one case normal wheat starch in the other finely ground wheat starch had been added, were ashed. The results are given in Table IV.

It is obvious that the addition of overground starch to normal flour increases the ash content of the gluten only slightly—certainly not enough to account for the great increase in ash found in gluten from overground flour. The small increase following the addition of 20% of ground starch may be merely due to difficulty of washing.

Effect of Overgrinding Upon the Acid Swelling of Gluten

Upson and Calvin (1915) have shown that if the wet gluten from different flours be placed in weak solutions of acids, the different glutens will swell in different degrees. To test the findings just reported, this method was used. Disks of gluten, freshly washed from flour before and after grinding, of as nearly uniform size and shape as possible, and weighing from 1.0 to 1.5 grams, were immersed in 0.04 normal acetic acid. The degree of swelling was determined by weighing again two hours later. The results for one of the flours used are presented in Table V. The other flour showed similar results. Experiments A, B, C, etc., represent different preparations of gluten from the same flour.

TABLE V

GRAMS OF 0.04 N ACETIC ACID SOLUTION ABSORBED PER GRAM OF MOIST GLUTEN

Normal flour		Flour overground 16 hours	
A	1.29	E	0.975
	1.28		0.94
B	1.68	F	0.72
	1.78		0.70
C	1.01	G	1.02
	0.99		0.64
D	0.85	H	0.87
	1.13		0.92
		E	0.95
			0.94
Average	1.25	Average	0.867

It is evident from the data of Table V that the rate of swelling in acid of gluten is lessened by overgrinding. Moreover, there was a decided difference in the appearance of the disks. The normal flour gluten disks were more swollen, puffy, slimy and runny, and therefore harder to handle. It was possible to tell the difference between the two sets of gluten disks by the most casual inspection.

Similar experiments were made with glutens washed from mixtures of uninjured wheat starch and normal flour, and from mixtures of severely ground wheat starch and normal flour. No marked difference in rate of swelling was observed between the two sets of glutens. Moreover, similar experiments were made using glutens of normal flours washed after 15, 30, 40 and 60 minutes standing of the dough for development. No difference in the rate of swelling was observed between them, indicating that the length of the "development" period does not notably affect the rate of swelling of the gluten when brought into acid.

IV. To What Extent Does Overgrinding Injure Baking Strength?

Bread was baked from the Turkey red flours overground in the pebble mill and from the corresponding normal flours in exactly the same way and at the same time. The same amount of water was used in both cases. The ground flour was doughed with difficulty and could not be kneaded as long as the control because of crumbling. The dough rose well, but after punching failed to rise again. The loaf was little more than half the size of the control. The crust was without bloom. The crumb was close and soggy with a grayish cast. This darker color as compared with the control was the more striking, as the flour was whiter than the unground control flour.

As, apparently, the overground flours required more water in doughing, the baking was repeated, using more water in doughing, 80% of water being used. As in the previous baking the dough rose again so little after punching, the dough was panned after the first

punch, proofed, and baked. The dough was difficult to handle because it was so sticky that it could not be molded properly. The loaf obtained by this procedure was far superior to the loaf obtained in the first baking, though it was still a miserable specimen, much smaller in volume, grayer, soggier, and with a much darker top crust than the normal loaf from the same flour. Incidentally it may be mentioned that the early stage of the fermentation was more rapid than in the normal—as would be expected from the fermentation experiments above recorded.

The effect of overgrinding upon the weaker flours was even more pronounced. In some cases they could not be doughed at all. They behaved as though they had hardly any gluten. It was impossible to bake bread from them.

The different samples of flour overground by Mr. Bailey were very kindly baked by Mr. Kress of the Sperry Flour Company, and the writers desire to express to him their real appreciation of this and many other courtesies. The results are given in Table VI. It is obvious that these flours were not sufficiently overground to injure them in regard to loaf volume or texture. The chief injury has been to the color; but in No. 18 and No. 20 this was not pronounced. Very remarkable is the effect upon the absorption and yield, which are markedly increased. In regard to absorption, therefore, overgrinding this flour had, qualitatively, an effect similar to the effect of the most severe overgrinding of the pebble mill.

TABLE VI

BAKING TESTS UPON AN OVERGROUND HARD SPRING PATENT FLOUR

	Passes through cloth	Color	Gluten color	Gluten quality	Absorption Per cent
Original flour	No. 10	Creamy white	Good	Very good	61
Ground flour	No. 15	Gray	"	"	63
"	No. 18	Slightly gray	"	"	63
"	No. 20	"	"	"	66
"	No. 21	Gray	"	"	64

	Passes through cloth	First expansion Minutes	Second expansion Minutes	Pan Minutes	Total Minutes	Loaves per bbl.	Loaf volume cc.
Original flour	No. 10	137	35	128	300	298	2080
Ground flour	No. 15	134	37	115	286	301	2000
"	No. 18	130	36	118	284	301	2000
"	No. 20	136	36	120	292	306	2030
"	No. 21	136	34	120	290	303	2020

Bread was also baked from Turkey red flour admixed with 20% of its own unground starch and from the same flour admixed with 20% of its own starch ground 118 hours in the pebble mill. The first mixture gave a good loaf, as was to be expected. The second be-

haved very much like the flour overground in the pebble mill above described, even to the more rapid initial fermentation. It produced a loaf similar in appearance and but little better. It is obvious that the addition of as much as 20% of overground starch to a normal flour greatly reduces its baking quality.

Discussion

On the evidence above presented it is obvious that severe overgrinding reduces the baking strength of flour and at the same time increases its water absorption. Therein overgrinding differs from other processes that reduce the baking strength of flour, for nearly all, if not all, decrease absorption if they reduce baking strength at all. Indeed, some cereal chemists define "baking strength" as the amount of water a flour is capable of absorbing.

It is also obvious, on the evidence above presented, that most—sometimes perhaps all—of the increase in absorption is due to injury to the starch granules, which makes them swell by absorbing water and disperse more or less. This must necessarily be the case for, as has been shown, the gluten from some of the flours overground in the pebble mill has a materially reduced water capacity. Yet the flour from which it was obtained has an increased absorption. This can be accounted for only if the injured starch granules absorb more than enough additional water to compensate for the reduced water capacity of the gluten.

Some evidence is also presented that in moderately overground flour the gluten also contributes to the increased absorption. Thus the character of the gluten washed from the overground hard spring flour received from Mr. Bailey gave rather higher wet gluten yields and the gluten had a rather higher protein content than in the case of the same flour before overgrinding. The evidence is by no means conclusive for it is based upon a single flour. It is proposed to repeat the experiment upon a series of different flours, for it is perhaps a matter of considerable practical importance to determine whether moderate overgrinding increases absorption regularly. If it does, it is conceivable that by carefully regulated grinding it may be possible to increase absorption without adversely affecting flour. It may be possible to break up the clumps of gluten, thereby exposing a greater surface to water absorption and facilitating imbibition, without too much injury to the starch. This might become a matter of technical importance.

Evidence has also been presented that very severe overgrinding affects the gluten very detrimentally. The evidence is not conclusive,

for there is always the possibility that the injured starch rather than the grinding process affects the gluten. However, it is hard to see how injury to the starch can affect the ash content of the gluten or affect the expansion in the oven of a lump of washed gluten which hardly contains any starch. While it must be admitted that the water capacity of washed gluten is not a safe basis for the drawing of conclusions concerning gluten quality because the method is not very exact, nevertheless reasonable checks were obtained and all the severely overground flours show more or less diminution in water capacity as compared with normal. This evidence is in harmony with the appearance and feel of the glutens. Finally, from the most severely overground flours no gluten at all could be washed. While, perhaps, no single piece of the evidence here presented by itself proves that grinding in itself injures gluten directly and not indirectly through the effect of the presence of injured starch, still the cumulative evidence is very strong and the conclusion seems warranted that overgrinding, if it be severe, injures the gluten as well as the starch.

How are we to conceive of the mode of action of overgrinding? This question, so far as it concerns starch, will be treated elsewhere. Only certain possible explanations of the effect upon gluten will be considered here. The simplest explanation, namely that overgrinding divides gluten so finely that it becomes colloidally dispersed like insoluble substances that are converted into suspensoids by passage through a colloid mill, is untenable. The microscope shows that the gluten is not converted into an impalpably fine dust. That micellae or small colloid aggregates are mechanically subdivided, there is no evidence whatever.

There are several hypotheses that may be advanced with greater appearance of reason. One is that the gluten in overground flour, having a much larger surface exposed to the water used in doughing, imbibes water more rapidly and in the fully imbibed state coalesces much less readily than less perfectly hydrated gluten. Hence the loss of quality.

A second hypothesis is that gluten as it occurs in the wheat berry has a definite anatomical structure and that it is the disintegration of this structure which results in loss of quality. That gluten has histological structure cannot be doubted, for it is part of a seed. It cannot be assumed that in flour glutenin and gliadin are uniformly and evenly mixed with one another as the salt and water molecules are mixed in brine. It is not impossible that the distribution of the two

proteins is far less uniform. Possibly one forms a reticulum in which the other is contained. If overgrinding destroys such a structure, it is to be assumed that it would affect gluten quality.

A third hypothesis is that in gluten, as it occurs in the wheat berry, there is crystalline material which becomes amorphous when ground. That grinding has such an affect upon inorganic crystalloids, has been shown by Ray (1922) (1923). It is not a far-fetched idea that some of the protein of the wheat endosperm may be in crystalline form. Protein crystals are very common occurrences in the endosperm of many seeds. The wheat berry endosperm is known to contain protein granules, and these may well be sphaeroliths, or at least be constructed in some manner whereby the molecules have an orderly arrangement. If this be the case, the effect of overgrinding may well be the result of a conversion of crystalline into amorphous matter.

A number of other hypotheses might be advanced to explain the observations recorded in this paper. This is not the place to go into all the theoretical possibilities. The writers have in progress investigations concerning the effect of fine grinding upon the physical properties of various organic colloids such as starch, gelatin, and agar, which have shown that such treatment greatly modifies their behavior. The results will be reported elsewhere. It is hoped that they will help to explain the effects of overgrinding upon flour.

Summary

Overgrinding flour injures the starch granules so that a part of the starch swells and disperses when the flour is doughed.

One result is an increase of the cold water extract from dispersed starch. Another is increased diastatic starch conversion in the flour from which an increased initial rate of fermentation follows.

Severe overgrinding injures flour for baking purposes despite increased absorption due to swelling of starch granules. Evidence is presented that it also injures the gluten.

Moderate overgrinding may injure the starch granules without much affecting the gluten. In the case of one flour examined, absorption was appreciably increased without material effect upon the baking strength.

The facts presented in this investigation point to the possibility of modifying to some degree the properties of flours by mere mechanical treatment. Studies looking to the exploration of these possibilities are in progress.

Literature Cited

Alsberg, C. L., and Perry, E. E.
1924. The effect of grinding upon starch and starch pastes. Proc. Soc. Exp. Biol. Med. Vol. 22, pp. 60-61.

Association of Official Agricultural Chemists.
1919. Methods of Analysis.

Bailey, C. H., and Johnson, A.
1924. Carbon dioxide diffusion ratio of wheat flour doughs as a measure of fermentation period. Cereal Chem., Vol I, pp. 293-304.

Collatz, F. A
1922. Flour strength as influenced by the addition of diastatic ferments. Amer. Inst. of Baking, Bul. 9.

Dill, D. B.
1925. The composition of crude gluten. Cereal Chem., Vol. 2, pp. 1-11.

Dill, D. B., and Alsberg, C. L.
1924. Some critical considerations of the gluten washing problem. Cereal Chem., Vol. I, pp. 222-246.

Hendel, J. and Bailey, C. H.
1924. The quality of gluten of flour streams as determined by the viscosity of water suspensions. Cereal Chem., Vol. I, pp. 320-324.

Huss, H.
1922. Untersuchingen über die Quellung der Stärkekörner. Arkiv Bot., Bd. 18, S. 7.

Le Clerc, J. A., Wessling, H. L., Bailey, L. H., and Gordon, W. O.
1919. Composition and baking value of different particles of flour. Operative Miller. Vol. 24, pp. 257-258.

Rask, O S., and Alsberg, C. L.
1924. A viscosimetric study of wheat starches. Cereal Chem., Vol. I, pp. 7-26.

Ray, R. C.
1922. Heat of crystallization of quartz. Proc. Roy Soc. (London), Series A, Vol. 101, p. 509.
1923. The effect of fine grinding on quartz (silver sand). Proc. Roy Soc. (London), Series A, Vol. 102, p. 604.

Richardson, J.
1914. Address before the convention of the Association of Operative Millers. Operative Miller, Vol. 19, p. 943.

Rumsey, L. A.
1922. The diastatic enzymes of wheat flour and their relation to flour strength. Amer. Inst. of Baking, Bul. 8.

Upson, F. W., and Calvin, J. W.
1915. On the colloidal swelling of wheat gluten. Jour. Am. Chem. Soc., Vol. 37, pp. 1295-1304.

Wanklyn, J. and Cooper, W. J.
1881. Bread analysis. A practical treatise on the examination of flour and bread. London.

Working, E. B.
1924. Lipoids, a factor influencing gluten quality. Cereal Chem., Vol. I, pp. 153-158.

IDENTIFICATION AND ESTIMATION OF THE ORGANIC ACIDS PRODUCED DURING BREAD DOUGH AND CRACKER DOUGH FERMENTATION[1]

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Introduction

Among the biochemical changes which take place during the fermentation of a dough with baker's yeast is an increase in hydrogen-ion concentration. Data presented by Bailey and Sherwood (1923) show that in a particular instance the hydrogen-ion concentration of a bread dough increased from 0.26×10^{-5} to 1.48×10^{-5} during a six-hour fermentation period. In a similar manner the acidity of cracker sponges increases markedly during progressive stages of fermentation. Because of the extended fermentation period to which cracker sponges are subjected, they become decidedly more acid than do bread doughs. Johnson and Bailey (1924) describe such a sponge in which the hydrogen-ion concentration increased from 0.153×10^{-5} to 18.400×10^{-5} during approximately 23 hours. Nine pounds of sodium bicarbonate was required to neutralize the acid-reacting materials which had effected this increase in hydrogen-ion concentration. As little definite knowledge was available concerning the exact chemical character of the acid-reacting material which accumulates in doughs during fermentation, research was undertaken to determine the identity of these materials.

At least three phenomena operating in a dough would conceivably effect an increase in hydrogen-ion concentration. These are: (1) the production of carbon dioxide by the yeast organism; (2) the liberation of acid-phosphate salts through the action of phytase on the phytin of the flour; and (3) the production of organic acids by yeast or bacteria present in the dough. Obviously these factors operate simultaneously to increase the intensity of the acid reaction of the dough. The rôle of each factor as it is related to the total quantity of acid-reacting material produced will be studied.

Experimental

Increase in Acidity of Flour-Water Suspensions Containing Yeast

Before undertaking a study of acid-reacting substances as they occur in bread and cracker doughs, some preliminary work was done on flour-water suspensions containing yeast. It was thought that in

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these more fluid media the conditions for the elaboration of the acid-reacting materials by the organisms responsible for them would more closely approach the optimum than would those in the stiffer bread or cracker doughs, hence such acid materials would accumulate and be present in sufficient quantity to make possible the development of a technique for their determination. Suspensions of flour, water and yeast were accordingly prepared. These suspensions contained 1,000 cc. of distilled water, 100 grams of flour, and one gram yeast. The flasks containing the suspensions were placed in a fermentation cabinet with the temperature maintained at 28° C. and fermentation was allowed to proceed for at least 24 hours. In certain instances the fermentation period was extended to 120 hours. The preliminary study was made and the technique developed on suspensions such as these.

Role of carbon dioxide in increasing acidity of flour-water suspensions containing yeast.—While it is to be expected that the carbon dioxide produced by yeast might have a measurable effect on the hydrogen-ion concentration of flour-water suspensions during early stages of fermentation, yet as the quantity of acid due to other factors increases, the effect of carbon dioxide production on both total acidity and hydrogen-ion concentration might be expected to decrease. In the first place the more acid suspensions would be unable to retain the carbon dioxide as efficiently as the almost neutral suspensions which existed at the beginning of fermentation. In the second place as carbonic acid is a weak acid it would be able to affect the hydrogen-ion concentration of suspensions containing it to a lesser and lesser degree as the maximum hydrogen-ion concentration of the carbonic acid solution is approached. These postulations were found to be correct and their substantiation as facts will appear during the course of this discussion.

In Table I is presented a summary, typical of other data which were obtained, showing the increase in titratable acidity and hydrogen-ion concentration (in terms of pH) of several flour-water suspensions containing yeast. Determinations of titratable acidity and hydrogen-ion concentration were always made on centrifuged portions of the suspension. It is to be noted that after the 48th hour there is little change in either titratable acidity or pH. There may even be a reason for a decrease in hydrogen-ion concentration after the 48th hour, although this is not indicated by the data in Table I. Apparently the organisms responsible for the development of acidity no longer operate to increase the acidity beyond a certain point. Johnson and Bailey (1924), however, have shown that after neutralization of the acids of fermentation

in a cracker sponge the dough again attains the same acidity as before neutralization, if sufficient time is given for the organisms to act; hence the high acidity temporarily inactivates but does not destroy the ability of the organisms in yeast-flour-water preparations to produce acid. The chief concern of this research, however, is with the estimation and identification of the acids produced and not so much with the mechanism of their production except as it is suggestive of their identity.

TABLE I

ACIDITY OF FLOUR-WATER SUSPENSIONS CONTAINING YEAST

Time fermented	N/14 NaOH per 25 cc. centrifuged suspension	pH
Hours	cc.	
0	1.10	5.90
24	7.65	3.33
24	8.20	3.27
24	6.95	3.42
48	16.40	3.20
48	15.00	3.27
48	15.60	3.22
120	16.60	....

As regards the part which carbon dioxide plays in the production of the relatively high acidity of the suspensions listed in Table I, little need be said except that it is very slight. Thus the suspension fermented for 24 hours and having a hydrogen-ion concentration equivalent to a pH of 3.33 had, after aspiration with hydrogen, a hydrogen-ion concentration equal to a pH of 3.37. Similar slight increases in hydrogen-ion concentration were found for several other suspensions after aspirating them with hydrogen or boiling for several minutes to expel the carbon dioxide.

Titrations (before and after aspiration) of flour-water suspensions fermented with yeast indicate that suspensions of the acidity shown in Table I contain very little carbon dioxide. Thus the original suspension requiring 7.65 cc. of N/14 sodium hydroxide for neutralization to phenolphthalein required an equal quantity of alkali for neutralization after aspiration with hydrogen. Determinations on several other suspensions gave similar results; hence the conclusion was reached that carbon dioxide plays little part in the final acidity of flour-water suspensions fermented at least 24 hours with yeast.

Role of phytase activity in increasing the acidity of flour-water suspensions.—The phytase of the flour might act on the phytin to liberate acid phosphate salts which would increase the acidity of the suspension both total and effective. Hence experiments were performed to distinguish the effects of a phytase-phytin phenomenon on increased

acidity from those of organic acids. Organic acids, if present, could be separated from an acid-salt solution by continuous extraction with ether. Consequently an extractor of the type devised by Dakin (1920) for the extraction of certain amino acids with butyl alcohol was obtained, and 300 cc. of a boiled solution of fermented flour-water suspension was extracted for three weeks. Preliminary trials had shown that extraction over a long period of time was necessary to remove any considerable quantity of the acid material. Before extraction, 25 cc. of the acid suspension required 15.5 cc. of N/14 sodium hydroxide for neutralization. After extraction for three weeks, a quantity of the suspension equivalent to 50 cc. of the original suspension required 2.50 cc. of the same alkali solution. The data in Table I indicate that 25 cc. of the original unfermented suspension required 1.10 cc. After extraction with ether, an aliquot of a suspension which had required 15.5 cc. of the same alkali, required but 1.25 cc. for neutralization; hence almost the total quantity of acids formed in a fermenting medium such as the one described is due to substances which are capable of extraction with ether. These substances are presumably organic acids.

Role of organic acids in increasing the acidity of flour-water suspensions fermented with yeast.—As organic acids elaborated in the metabolic processes of yeast, or bacterial activity, are likely to be the most important agents involved in increasing the acidity of flour-water suspensions fermented with yeast, a brief consideration of the acids likely to be encountered may be worth while. Several of the more recent theories of alcoholic fermentation postulate the formation of pyruvic acid as an intermediate product. Neuberg and Hildesheimer (1911) and Kostytschev (1912) state that pyruvic acid is fermented more readily than sucrose. Lebedev and Polonski (1917), however, state that pyruvic acid is fermented less completely to carbon dioxide and alcohol than sucrose; hence they do not believe that it occupies so important a place in the mechanism of yeast fermentation as do Neuberg and his co-workers. Neuberg (1922) and Fernbach and Schoen (1922-23) find that pyruvic acid can be fixed in fermenting media by adding some substance with which it will react. Neuberg used potassium bisulphite for this purpose, while Fernbach and Schoen used powdered chalk. Hence there is the possibility of pyruvic acid being formed, although it is unlikely that a substance so readily fermentable by yeast should accumulate and operate to increase the hydrogen-ion concentration. As a possibility, however, it should be considered and its determination and identification studied.

Neuberg and Kerb (1913) believe that pyruvic acid is formed in accordance with the mechanism of alcoholic fermentation which follows:

(1) The hexose molecule is split into two molecules of methyl glyoxal with loss of water

$C_6H_{12}O_6 \rightarrow 2CH_3CO\ CHO + 2H_2O$

(2) A portion of the aldehyde thus formed is converted to pyruvic acid and an equal portion to glycerol by a Cannizzaro reaction

$2CH_3CO\ CHO \rightarrow CH_2OH\ CHOH\ CH_2\ OH + CH_3CO\ COOH$

(3) The pyruvic acid thus formed is decomposed by a decarboxylase to give ethyl alcohol and carbon dioxide

$CH_3CO\ COOH \rightarrow CH_3CH_2OH + CO_2$

Neuberg (1922) states that if the optimum hydrogen-ion concentration for alcoholic fermentation exists in the medium, alcohol and carbon dioxide only will be formed in accordance with the foregoing mechanism. If the medium is more alkaline than the optimum, a side reaction occurs which results in the production of acetic acid. As the hydrogen-ion concentration of flour-water suspensions containing yeast varies considerably over a 24-hour fermentation period (from pH 5.9 to pH 3.3 in the case shown in Table I), it is likely that at certain stages of the fermentation process the hydrogen-ion concentration is far enough on the alkaline side of the optimum that the production of small quantities of acetic acid may result. Neuberg believes that the mechanism of acetic acid production involves the following reactions:

(1) Acetaldehyde, glycerol, and carbon dioxide are produced from the sugar by the yeast.

$2C_6H_{12}O_6 \rightarrow 2CO_2 + 2CH_3CHO + 2C_3H_8O_3$

(2) One molecule of the acetaldehyde thus formed is oxidized to acetic acid, the other is reduced to ethyl alcohol. The mechanism of this transformation is again the familiar Cannizzaro reaction.

$2CH_3CHO \rightarrow CH_3CH_2OH + CH_3COOH$

Another organic acid found quite generally in fermenting material is lactic acid, at one time considered an intermediate product in alcoholic fermentation. As it is not readily fermented by yeast, however, this theory of fermentation has been discarded. Lieben (1922) and Furth and Lieben (1922) state that yeast can utilize small quantities of lactic acid, especially if an atmosphere rich in oxygen is provided. No alcohol is produced in such media, the lactic acid which disappears being accounted for in carbon dioxide plus increase in tissue weight

of the yeast. In commercial bakers' yeast, however, the presence of organisms capable of producing lactic acid is quite certain, especially in view of the method of its manufacture, hence it is likely that these organisms may be responsible for the production of some of the acids found in fermented material. Fred, Peterson, and Stiles (1925), moreover, have shown that the cereal grains generally have associated with them a species of high acid-forming bacteria which produces laevo-lactic acid in its metabolism. In all probability, therefore, lactic acid will help to increase the acidity of fermenting media.

The presence of succinic acid in yeast-fermented materials has also been established. Ehrlich (1909) showed that this acid was produced through an oxidation of glutamic acid.

$$COOH{-}(CH_2)_2\underset{\displaystyle |\atop NH_2}{CH}{-}COOH + O_2 \rightarrow COOH\,(CH_2)_2\,COOH + CO_2 + NH_3$$

As the addition of nitrogen available to the yeast reduces the quantity of succinic acid produced, the yeast apparently utilizes the ammonia as its source of nitrogen.

In addition to acetic acid, other fatty acids, particularly the lower ones of the series, are likely to be produced in the media, owing to bacterial activity; hence this possibility must be considered. Butyric acid may be considered as typical of these and its presence or absence in fermented material must be established.

In determining the relative proportions of these acids which might be present in media prepared as indicated, a knowledge of their distribution between ether and water might be of significance. By determining the coefficient of distribution of the acids produced during the souring of milk, Van Slyke and Baker (1918) were able to ascertain the quantity of free lactic acid present in sour milk. Thus when a water solution of the organic acid is treated with ether and the mixture is shaken until the ether dissolves all the acid that it can, the concentration of acid in the ether layer divided by the concentration of acid remaining in the water layer, gives a factor which is constant at any one temperature for any solution of the particular acid, without regard to the relative volumes of ether and water. This constant is called the coefficient of distribution. The coefficients of distribution of the organic acids indicated as likely to be present in fermenting material were determined and the results are shown in Table II. Difficulties were encountered in the work with lactic acid because of the property of this acid to react with itself with the formation of the anhydride. By boiling with an excess of barium hydroxide, the anhydride is decomposed. Free lactic acid is then obtained by precipitat-

ing the barium with dilute sulfuric acid and extracting the lactic acid with ether. The coefficient of distribution of lactic acid can then be determined in the usual way.

TABLE II

DISTRIBUTION OF SEVERAL ORGANIC ACIDS BETWEEN WATER AND ETHER AT 25° C.

	Standard alkali solution per 25 cc. organic acid solution Ether layer	Water layer	Coefficient of distribution
Organic acid	cc.	cc.	E/W
Acetic	15.20	28.40	.5352
Butyric	85.50	12.15	6.3374
Lactic	4.15	52.00	.0798
Pyruvic	18.50	92.25	.2005
Succinic	7.50	47.25	.1587

The data in Table II indicate that considerable difference exists between the coefficients of distribution of the acids considered. The polar nature of the hydroxyl and carboxyl groups of lactic acid tends to increase its concentration in the water layer and the non-polar nature of the organic radical of butyric acid tending on the other hand to increase its concentration in the ether layer.[2] Assuming that there are only two acids present in a water or ether solution it would be possible to determine the quantity of each by a simple determination of the coefficient of distribution if the identity of the acids and their coefficients of distribution are known, as substances distribute themselves independently of each other unless they react with substances in the systems containing them. In working with fermented flour-water suspensions it was not possible to determine accurately the concentration of acids present in the ether layer and in the flour-water suspension layer, because of the alkali-binding property of the substances in the flour-water suspension. For this reason centrifuged suspensions of the fermented material were extracted with ether for several days and the coefficients of distribution were determined on these ether extracts. In Table III are shown the results. In all the experiments except No. 6 the coefficient of distribution is fairly constant. It is probable, as will be shown later, that the longer fermentation period in this instance results in the production of different proportions of the several acids.

The data presented in Tables II and III are of significance in considering the probable combination of acids responsible for the increas-

[2] It is suggested that the determination of the distribution of a series of substances between polar and non-polar solvents may serve as a means of establishing the relative polarity of organic groups. For example pyruvic and lactic acids are the same except for the groups on the α carbon atom. From the data in Table II it is noted that the relative concentration of lactic acid in the water layer is considerably greater than the relative concentration of pyruvic acid in the water layer. This would indicate that the group on the α carbon atom of lactic acid is more polar than that on the same carbon atom of pyruvic acid.

ing acidity in fermenting media. As the coefficient of distribution of the unknown mixture of acids is approximately 0.1120 it is not likely that butyric acid is present in any considerable quantity. The presence of even small quantities of this acid in the mixture of acids extracted with ether would give a distribution ratio much higher than the value actually found. On the other hand, lactic acid is quite likely to be present because the distribution ratio found for the unkown mixture of acids is intermediate between the value for lactic acid and that for any other acids considered in Table II. The problem, therefore, consists in determining which acid—acetic, succinic or pyruvic—is present with lactic, and further to substantiate the presence of lactic acid and the absence of butyric acid in the mixture of acids extracted with ether.

TABLE III

Distribution of Organic Acids Extracted from Fermented Material Between Ether and Water at 25° C.

Expt.	Fermenting period	Standard alkali solution per 25 cc. organic acid solution Ether layer	Water layer	Coefficent of distribution
No.	Hours	cc.	cc.	E/W
1........	24	1.10	9.90	.1111
2........	48	1.80	17.80	.1011
3........	24	1.50	13.15	.1140
4........	48	1.00	8.05	.1242
5........	48	2.10	18.10	.1160
6........	120	2.35	11.60	.2025

Pyruvic acid may be detected in several ways. A colorimetric test depending on the formation of indigo blue [Baeyer and Drewsen (1882)] by interaction between o-nitrobenzaldehyde and pyruvic acid in alkaline solution was found to be the most sensitive for the determination of pyruvic acid in the small quantities likely to be encountered in fermented material. This test was made on several dilutions of pure pyruvic acid in water and was found to be sensitive to about 1 part acid to 5,000 parts water. In order to separate the indigo blue from the colored materials in the reaction mixture, the mixture was extracted with chloroform. Blue in the chloroform layer would therefore indicate the presence of pyruvic acid and the intensity of color would indicate the relative quantity. Pyruvic acid could not be identified in the mixture of acids extracted from fermented media with ether, altho the extract was concentrated to one-tenth the volume of the suspension extracted. This indicates that pyruvic acid, if present, is less than 1 part of the acid to 50,000 parts of the fermented material; hence the rôle of pyruvic acid in increasing the acidity of the fermenting mixture is negligible.

Small quantities of succinic acid can be detected by following the procedure of Bordas, Joulin, and Raczkowski (1898). This method depends on precipitation of insoluble silver succinate from neutral solutions containing the succinate radical when silver nitrate solution is added. In the use of this method care must be taken that no substances are present which give precipitates with silver nitrate. At great dilutions the quantity of succinate present is indicated by the relative turbidity of preparations containing suspended silver succinate. By the application of this method to solutions containing known quantities of sodium succinate, it was possible to detect the succinate radical at dilutions as great as 1 part of succinic acid to 100,000 parts of water. When neutral aqueous solutions of the acids of fermentation were thus tested, no trace of succinic acid was observed. This observation eliminates the possibility of the accumulation of succinic acid being responsible for increase in the acidity of fermenting material.

The elimination of pyruvic and succinic acids leaves only acetic and lactic to be considered as acids which effect changes in acidity coincident with fermentation. Acetic acid is volatile, while lactic is relatively non-volatile; hence it should be possible to separate the two by distillation. An aqueous solution of the ether-extracted acids was accordingly distilled under reduced pressure and the distribution coefficients of the volatile and the non-volatile acids were determined. The coefficient for the volatile acid was 0.4976 while that of the non-volatile acid was 0.0900. The addition of water and redistillation of the non-volatile residue reduced the distribution coefficient to practically that of pure lactic acid. Without question these data indicate the presence of considerable quantities of lactic acid in flour-water suspensions fermented with yeast. In determining the coefficient of distribution, it was necessary to decompose the anhydride of lactic acid by treatment with barium hydroxide and proceed as in determining this coefficient for pure lactic acid.

The coefficient of distribution of the volatile acids was found to be 0.4976 in the instance cited. This value approximated quite closely the coefficient for pure acetic acid, hence it was possible that traces of lactic acid which had distilled would lower the distribution coefficient. If this were the case, it could be proved that lactic and acetic were the only acids present in the fermenting media which were used. A method was accordingly sought to identify another acid present in the volatile material, if such were the case. As several investigators have shown that small quantities of lactic acid distill when solutions containing it are subjected to distillation, the presence of this acid in the distillate seemed quite likely. Von Fürth and Charnass (1910) developed

a method for determining lactic acid which seemed to have particular advantages. This method depends on the oxidation of lactic acid to acetaldehyde. The acetaldehyde is allowed to react with potassium bisulphite, the quantity of bisulphite required being estimated by titrating aliquots of the solution with iodine before and after the reaction with the aldehyde. If the oxidizing solution is added too rapidly, however, some of the lactic acid is oxidized to acetic acid, hence low results are usually obtained by this method. The oxidizing reactions are shown in the following equations:

(1) $CH_3CHOH\ COOH+O=CH_3CHO+CO_2+H_2O$

(2) $CH_3CHOH\ COOH+2\ O=CH_3COOH+CO_2+H_2O$

If the acid is identified as acetic by determining its coefficient of distribution between ether and water, the decomposition of lactic acid to form any of the substances shown in the equations will not affect the results. The aldehyde and carbon dioxide can be readily removed by aeration, and as the acetic acid is identical with the acid in question, its presence, by increasing the concentration, will assist rather than interfere.

According to the method of von Fürth and Charnass, the solution under investigation is acidified with sulfuric acid and placed in a distillation flask connected to a condenser. The delivery tube of the condenser is so arranged that it extends into a graduated cylinder containing a few cubic centimeters of distilled water, the end of the delivery tube being below the surface of the water. The distillation flask is fitted with a separatory funnel by means of which potassium permanganate solution can be introduced at the proper time. In order to remove all oxygen from the system, the solution is boiled until no more air bubbles leave the delivery tube. Then the permanganate is allowed to drip into the boiling solution at such a rate that the color disappears before the next drop strikes the surface. When the solution is permanently discolored, the permanganate supply is stopped and the solution boiled until several hundred cubic centimeters of distillate is obtained.

A considerable quantity of the distillate obtained from an aqueous solution of the substances extracted with ether from flour-water suspensions fermented with yeast, was neutralized with sodium hydroxide and evaporated to dryness on a water bath in order to remove any alcohol which might be present. The residue was taken up with water and treated according to the method of von Fürth and Charnass. The distillate from this process should contain (1) the acetic acid present in the original solution, (2) the acetic acid resulting from the oxidation of lactic acid, and (3) acetaldehyde and carbon dioxide resulting

from the oxidation of lactic acid. The last two were removed by aeration and the coefficient of distribution was determined on the aerated solution. A value of 0.5308 was obtained. This indicates that acetic acid is the only so-called volatile acid produced in flour-water suspensions fermented with yeast. If propionic or butyric acids had been present, a considerably higher distribution coefficient would have resulted, because they both distill more readily from aqueous solutions than does acetic acid.

As acetic acid is volatile, it is possible to obtain it directly from the fermented suspension by distillation under reduced pressure. Fermented flour-water suspensions were accordingly distilled. When the distillation had proceeded almost to dryness, additional water was introduced and the suspension distilled a second time. Water was usually added a third and a fourth time and the corresponding distillates were obtained. Five hundred cubic centimeters of the original suspension was usually used in this work and 250 cc. of water was added for each distillation. In one case the total titratable acidity of 500 cc. of a 24-hour fermented suspension was equivalent to 148.4 cc. of N/14 alkali. The titratable acidity of each of the distillates obtained in succession was equivalent to 3.2, 1.15, 0.70, and 0.50 cc. of standard alkali, respectively. For a suspension fermented for 48 hours, the titratable acid was about twice as great. In one case the total titratable acidity of 500 cc. of a 48-hour suspension was equivalent to 240 cc. of the standard alkali. The volatile acid obtained in four successive distillates was equivalent to 7.10, 2.30, 1.50, and 1.05 cc. of the standard alkali solution. From these data it is evident that the quantity of volatile acid produced in a fermenting flour-water suspension is relatively small, even when fermentation is allowed to proceed as long as 48 hours.

As lactic acid is slightly volatile it is obvious that small quantities of this acid are also contained in the distillate. Lactic acid may be determined according to the method of von Fürth and Charnass, which has already been described. In carrying out these determinations, the four neutralized distillates were combined, evaporated almost to dryness on a water bath, and the lactic acid was estimated from the quantity of acetaldehyde produced after oxidation of the mixture in acid permanganate solution. The total volatile acids minus the lactic acid thus estimated gives the true quantity of acetic acid produced. The true quantities of acetic acid present in several flour-water suspensions fermented with yeast for varying periods of time, are given in Table IV. In all cases the identity of the acid as acetic was established by a determination of the coefficient of distribution. The method of

Duclaux used by Dox and Neidig (1913) and Hammer and Sherwood (1923) to determine the volatile acids produced by organisms present in silage and in starters, was tried but did not give satisfactory results because of the large proportion of lactic acid in the acids of fermentation.

TABLE IV

Acetic Acid Content of Several Flour-Water Suspensions Fermented with Yeast for Different Periods of Time

Time fermented	N/14 alkali per 500 cc. suspension	N/14 alkali per total organic acids produced in 500 cc. of suspension	N/14 alkali per distillate from 500 cc. of suspension	N/14 alkali per lactic acid in distillate	N/14 alkali per true acetic acid from 500 cc. suspension	Acetic acid in total organic acid
Hours	cc.	cc.	cc.	cc.	c.c	Per cent
0	28.0	0	0	0	0	0
24	153.0	125	7.00	2.25	4.75	3.80
48	300.0	272	11.70	2.25	10.45	3.84
72	306.0	278	21.30	2.60	18.70	6.72
120	320.0	292	89.00	2.33	86.67	29.68

From the data presented, it appears that lactic and acetic are the only organic acids produced in flour-water suspensions fermented with yeast. Of these two, lactic acid is produced in much the greater quantity. At the end of 48 hours of fermentation, acetic acid constitutes less than 4 per cent of the total acid present, lactic acid about 90 per cent, and the remainder is due to the original acid reaction of the suspension plus a slight increase in acidity due perhaps to phytase-phytin activity. Thus, in Table IV, the acidity equivalent to 28 cc. of N/14 alkali at zero fermentation was determined, using 500 cc. of a flour-water suspension to which a few cc. of toluene had been added and the suspension allowed to stand for 24 hours.

Table IV also shows that after the 48th hour the quantity of acetic acid produced increases, although there is only a slight increase in the quantity of total acid. The low rate of acid production in the later stages of fermentation is probably due to the inability of the organisms responsible for acid production to continue their normal metabolic processes in the highly acid media. The reasons for the increase of acetic acid in media which are allowed to ferment for extended periods of time are unknown. As the total acidity remains practically the same, some of the lactic acid must disappear. Fürth and Lieben (1922) state that yeast cells may utilize lactic acid in their metabolism; a part of the acid appearing as carbon dioxide and the rest as increase in body weight, no alcohol being formed. This may account for the disappearance of lactic acid. It is suggested that in some manner the alcohol present in the media or that which might be formed in such

acid media is oxidized to acetic acid. No evidence, however, is at hand to support this idea.

Increase in Acidity of Cracker Sponges Fermented with Yeast

Role of carbon dioxide in increasing the acidity of cracker sponges fermented with yeast.—It is customary in cracker manufacture to prepare either a stiff or a soft sponge and to allow this to ferment for a period varying from 18 to 24 hours. At the end of this sponge period the acids produced during fermentation are neutralized with sodium bicarbonate, fresh flour is added, and after an additional fermentation period varying from 2 to 4 hours, the dough is baked into crackers. Johnson and Bailey (1924) have followed the changes in hydrogen-ion concentration and in acidity which result during cracker dough fermentation. Their data show that in the later stages of fermentation, the hydrogen-ion concentration of sponge suspensions was approximately equivalent to that of the fermented flour-water suspensions described in the previous section. Their data also show that carbon dioxide had little effect either on the hydrogen-ion concentration or on the titratable acidity of the sponge suspension, probably because it was not present in any considerable quantity, owing to the high hydrogen-ion concentration of the suspension. Even had it been present it is not likely that it would have affected the hydrogen-ion concentration, as carbonic acid is a weak acid. These observations as well as others obtained in connection with this work, indicate that the rôle which carbon dioxide plays in the final acidity of a cracker sponge is small.

Role of phytase-phytin activity in increasing the acidity of cracker sponges fermented with yeast.—As phytase activity was responsible for so slight a quantity of the acid-reacting material produced in fermented flour-water suspensions, it would be expected that such activity is just as small in cracker sponges. This was found to be the case. The original acidity of 25 cc. of the flour-water suspension used in this work was equivalent to 1.10 cc. The acidity of a corresponding quantity of ether-extracted sponge solution was never greater than an acidity equivalent to 1.50 cc. of N/14 alkali. Thus it is evident that organic acids are responsible for the greater part of the acidity developed in a cracker sponge during fermentation.

Role of organic acids in increasing the acidity of cracker sponges fermented with yeast.—As lactic and acetic were found to be the only acids present in flour-water suspensions fermented with yeast, it was likely that they would also be the only ones present in fermented cracker sponges. Experimental methods developed in a

foregoing section showed this to be the case. It remained, therefore, to determine the relative quantities of lactic and acetic acids present. Portions of cracker sponge were accordingly suspended in water in such proportion as to make the flour-to-water ratio equivalent to the ratio used in the work on flour suspensions. These preparations were subjected to distillation under reduced pressure according to the method previously described, in order to determine the quantity of acetic acid present. Five hundred cc. of one such preparation had a total acidity equivalant to 151 cc. of N/14 alkali. The hydrogen-ion concentration of this suspension was equivalent to a pH of 4.02. The true acetic acid present in the distillate obtained was equivalent to 27 cc. of N/14 alkali. This is nearly 22 per cent of the organic acid of the sponge. In working with several cracker sponges it was found that the percentage of acetic acid varied from 5 to 22 per cent of the organic acid. The reasons for this variation are unknown to the writer, unless it be assumed that sponges or flours vary sufficiently in their bacterial content as regards both species and number to result in the varying proportions of acids found. It should be noted that the material on which these determinations were made was prepared from cracker sponges obtained from a commercial biscuit factory over a period of several weeks, while the flour used in the work on flour-water suspension was taken from a small sample stored in a tight can.

In order to obtain further evidence that the non-volatile acid consisted entirely of lactic acid, the residues after distillation were taken up with water and extracted with ether. An aqueous solution of the extracted acids was prepared, and after decomposing any anhydride which might be present the coefficient of distribution was determined. Several such determinations gave distribution coefficients which varied from 0.088 to 0.092. It is probable that small quantities of acetic acid not removed by the four distillations were present and hence the resultant distribution coefficients were slightly higher than the value for pure lactic acid, 0.080.

A portion of the sponge suspension of total acidity equivalent to 151 cc. of N/14 alkali was extracted with ether and the coefficient of distribution of the extract determined. The value of the coefficient was found to be 0.1819. This gives confirmatory evidence that the mixture of organic acids extracted from this cracker sponge suspension contained about 22 per cent of acetic acid and 78 per cent of lactic acid.

Increase in Acidity of Bread Doughs Fermented with Yeast

Role of carbon dioxide in increasing the acidity of bread doughs fermented with yeast.—Bailey and Sherwood (1923) fol-

lowed the march of hydrogen-ion concentration in bread doughs but made no effort to determine the rôle of the several factors responsible for it. As bread doughs are not fermented for so long a time as the flour preparations already discussed, it is probable that carbon dioxide plays a more prominent part in affecting the acidity of the bread dough than in affecting the acidity of cracker sponges or of flour-water preparations. The data in Table V show this to be a fact. In dough No. 1 the original pH was 5.76. Five hours later, or just before the dough was baked, the pH had fallen to 5.24. After aspirating a suspension of the 5-hour fermented dough the pH was 5.58, which indicates that carbon dioxide was responsible for more than half the fall in pH value which resulted during fermentation.

TABLE V

HYDROGEN-ION CONCENTRATION (IN TERMS OF pH) AND TITRATABLE ACIDITY OF BREAD-DOUGH SUSPENSIONS

	Dough No. 1		Dough No. 2	
	pH	Titratable acidity	pH	Titratable acidity
1. Fresh dough	5.76	1.10	5.75	1.10
2. Fresh dough after 5 hours	5.24	3.36	5.18	3.50
3. No. 2 after aspiration with H_2	5.58	1.60	5.48	1.70
4. Baked bread	5.43	....	5.38	....
5. No. 4 after aspiration with H_2	5.56	....	5.48	....

Bailey and Sherwood also noted that the baked bread always had a lower hydrogen-ion concentration than the dough from which it was baked. The fact that aspiration of the dough suspension effected the same result suggested that perhaps the loss of carbon dioxide was responsible for the decrease in hydrogen-ion concentration. A bread suspension was accordingly prepared which contained approximately the same quantity of solid material in relation to solvent as was used in preparing the dough suspension. In dough No. 1 the hydrogen-ion concentration of the bread (in terms of pH) was 5.43, a value intermediate between that of the aspirated and the unaspirated dough suspension. Upon aspiration of the bread suspension to remove carbon dioxide which might be present, the hydrogen-ion concentration of the bread suspension became the same as that of the aspirated dough suspension. These data indicate that during the baking process loss of carbon dioxide from the baking dough results in a decrease in hydrogen-ion concentration. The baking, however, does not remove all the carbon dioxide, hence the hydrogen-ion concentration of the baked bread is intermediate between that of the unaspirated and the aspirated dough suspension.

The carbon dioxide in the dough suspension also has a measurable effect on the titratable acidity. Suspensions of the dough having

a 1 to 10 flour-water ratio were prepared and their titratable acidity was determined. The acidity of 25 cc. of suspension prepared from a fresh dough was equivalent to 1.10 cc. of N/14 alkali. Five hours later the acidity (in terms of the same units) had increased to 3.36 The removal of the carbon dioxide by aspiration reduced the acidity to 1.60. From these data it appears that the greater part of the acidity developed during the fermentation of a bread dough is due to the presence of carbon dioxide.

Role of organic acids in increasing the acidity of bread doughs fermented with yeast.—As the data in Table V indicate that phytase-phytin phenomena plus organic acid production play such a small rôle in increasing the acidity of bread doughs, and as it has already been shown that the increase in acidity due to phytase-phytin activity is very slight even under conditions more favorable than those in a bread dough, a discussion of this factor will not be given in connection with bread doughs.

The small quantity of organic acid present in the dough tends to make its determination difficult. The same methods were used, however, as were used in the previous work except that the dough suspensions contained as large a quantity of solid material as was possible. Such suspensions were extracted with ether in the usual manner and pyruvic, butyric, and succinic acids were shown to be absent. The coefficients of distribution of the extracted acids were determined and were found to vary from 0.1900 to 0.2100, indicating that about 25 per cent of acetic acid was contained in the extract. By distilling several suspensions under reduced pressure and determining the acetic acid in the distillate, this acid was shown to be present in the same proportion as indicated by distribution coefficients of the mixed acids. By extracting the residue after distillation several times with ether, and determining the coefficient of distribution of the extracted acid, lactic acid was shown to be the only non-volatile acid present. Thus, also, lactic and acetic are the only organic acids in bread doughs, and of the two, lactic acid is present in much greater quantity.

Discussion

The final acidity of flour-water suspensions and cracker sponge suspensions is due almost entirely to organic acids which have accumulated during fermentation. The titratable acidity of these materials may be practically the same at the end of a 24-hour fermentation period. It appears, however, that flour-water suspensions are likely to contain a greater proportion of their organic acids as lactic acid. The experimental data indicate this as well as certain indirect evidence

which might be pointed out. In Table I, the 500 cc. of the first suspension tabulated as being fermented 24 hours had a total acidity equivalent to 153 cc. of standard alkali solution and a hydrogen-ion concentration equal to a pH of 3.33. A cracker sponge suspension of the same flour-water concentration was discussed in which the total acidity of an equal quantity of suspension was equivalent to 151 cc. of the same alkali. The hydrogen-ion concentration of the cracker sponge suspension was equal to a pH of 4.02. Now it is known that lactic is a stronger acid than acetic, hence the greater proportion of lactic acid in the fermented flour-water suspension results in a higher hydrogen-ion concentration, although the total acidity of the two suspensions is practically the same.

It is to be noted also that the quantity of acetic acid present in the organic acids of the cracker sponge varied between 5 and 22 per cent. This fact helps to account for the varying quantity of sodium bicarbonate it was found necessary to add to cracker sponges of the same hydrogen-ion concentration in order to neutralize the acids of fermentation. As cracker sponges contain varying quantities of acetic and lactic acids, a determination of the titratable acidity should give a much more accurate indication of the quantity of sodium bicarbonate to be added than a determination of the hydrogen-ion concentration. In actual practice this was found to be the case, although the reason given was not then known to be a contributory factor.

While the production of carbon dioxide and its presence in the fermenting media undoubtedly play a part in the increasing acidity during the early stages of fermentation in cracker sponges and flour-water suspensions, their importance, so far as the final acidity is concerned is insignificant. In bread doughs, however, carbon dioxide plays the most important rôle of any of the factors responsible for the increasing acidity. Cracker sponges or flour-water suspensions, if investigated at the proper stage of fermentation, would probably show the same properties as regards the effect of carbon dioxide on acidity as were found with bread doughs.

The proportion of lactic acid and acetic acid appears to be different in different types of media. Lactic acid is formed almost exclusively in the flour-water suspensions fermented for short periods of time, while acetic acid production seems to increase with the increasing viscosity of the medium. The explanation of these results must lie in probable differences in rate of metabolic activity of the species of organisms responsible for the production of each acid, these differences in metabolic activity being due to differences in the media in which the organism operates.

The activity of phytase in flour preparations is not an important factor in accounting for the changes in acidity which occur. The work of Bailey and Peterson (1921) shows the hydrogen-ion concentrations of flours extracted with water for varying periods of time to be the same. The titratable acidity of the extract increases slightly, however, with an increasing period of extraction.

For all the work which has been described, a flour from a single source was used except, of course, that in the cracker sponges obtained from a biscuit factory. It is possible that if several flours had been used, other types of acids would have been formed owing to the presence of organisms capable of elaborating them or because the proportions of acids formed would differ. Investigation along these lines, however, will be left for the future.

Summary and Conclusion

Factors involved in the increase of the acidity of flour-water suspensions, cracker sponges, and bread doughs fermented with yeast are (1) carbon dioxide produced by the yeast, (2) acid phosphate salts produced by phytase-phytin phenomena, and (3) organic acids produced by the yeast or other organisms present in the media.

Carbon dioxide produced by yeast and acid phosphate salts resulting from phytase activity have very slight effect on the final acidity of flour-water suspensions and cracker sponges fermented with yeast.

Carbon dioxide present in a bread dough is the most important single factor affecting its hydrogen-ion concentration and titratable acidity.

Lactic and acetic are the only organic acids found in flour preparations fermented with yeast.

Lactic acid occurs in flour-water suspensions to the extent of from 70 to 96 per cent of the organic acid present. The remainder of the organic acid is acetic acid. The proportion of acetic acid appears to increase when the fermentation period is increased.

Lactic acid occurs among the acids of a cracker sponge in quantities varying from 78 to 95 per cent of the organic acid. The rest of the organic acid present in a cracker sponge is acetic acid.

Lactic acid occurs in bread dough to the extent of about 75 per cent of the organic acid present and acetic acid constitutes the remaining 25 per cent.

Bibliography

Baeyer, A., und Drewsen, U.
1882. Darstellung von Indigblau and Orthonitrobenzaldehyd. Ber. d. d. chem. Gesell., Bd. 15, S. 2856-2864.

Bailey, C. H., and Peterson, A. C.
1921. Studies of wheat flour grades. II. Buffer action of water extracts. Jour. Ind. Eng. Chem., Vol. 13, pp. 916-918.

Bailey, C. H., and Sherwood, R. C.
1923. The march of hydrogen-ion concentration in bread doughs. Jour. Ind. Eng. Chem., Vol. 15, pp. 624-627.

Bordas, Joulin, and Raczkowski
1898. The estimation of succinic acid in the presence of tartaric and lactic acids. Chem. News, Vol. 78, pp. 18-19.

Dakin, H. D.
1920. Amino acids of gelatin. Jour. Biol. Chem., Vol. 44, pp. 499-529.

Dox, A. W., and Neidig, R. E.
1912. The volatile aliphatic acids of corn silage. Iowa Agr. Exp. Sta., Res. Bul. 7.

———
1913. Lactic acid in corn silage. Iowa Agr. Exp. Sta., Res. Bul. 10.

Ehrlich, F.
1909. Ueber die Entstehung der Bernsteinsäure bei der alkoholischen Gärung Biochem. Zeitsch., Bd. 18, S. 341-343.

Fernbach, A., and Schoen, M.
1922. L'acide pyruvique dans la fermentation alcoolique. Compt. rend. soc. biol., t. 86, pp. 15-18.

———
1923. Se forme-t-il de l'acide lactique dans la fermentation alcoolique? Compt. rend. soc. biol., t. 89, pp. 475-477.

Fred, E. B., Peterson, W. H., and Stiles, H. R.
1925. The biochemistry of the granulated lactic acid bacteria from cereals. Jour. Bact., Vol. 10, pp. 63-78.

Fürth, O. von, and Charnass, D.
1910. Über die quantitative Bestimmung der Milchsäuer durch Ermittlung der darans abspalten Aldehydmenge. Biochem. Zeitsch., Bd. 26, S. 199-220.

Fürth, O. von., and Lieben, F.
1922. Über Milchsauerzerstarung durch Hefe und durch Blutzellen. Biochem. Zeitsch., Bd. 128, S. 144-168.

Hammer, B. W., and Sherwood, F. F.
1923. The volatile acids produced by starters and by organisms isolated from them. Iowa Agr. Exp. Sta., Res. Bul. 80.

Johnson, A. H., and Bailey, C. H.
1924. A physico-chemical study of cracker dough fermentation. Cereal Chemistry, Vol. I, pp 327-410.

Kostytschev, S.
1912. Ueber Alkoholgärung. Ueber die Bildung von Acetaldehyd bei der alkoholischen Zuckergärung. Zeitsch. physiol. Chem., Bd. 79, S. 130-145.

Lebedev, A. N.
1917. L'acide lactique est-il un produit intermediare de la fermentation alcoolique. Biochem. Jour., Vol. 11, pp. 189-196.

Lebedev, A. N., and Polonski, A. N.
1917. The fermentation of pyruvic acid in the presence of sugar. Jour. Russ. Phys. Chem., Soc., Vol. 49, pp. 328-344.
C. A., Vol. 17, p. 3199. (Original not seen.)

———
1917. The fermentation of glyceric and pyruvic acids. Jour. Russ. Phys. Chem. Soc., Vol. 49, pp. 344-357.
C. A., Vol. 17, p. 3199. (Original not seen.)

Lieben, F.
1922. Über Zerstärung von Milchsäure durch Hefezellen. Oester. Chem. Zeit., Bd. 25, S. 87-90.

Neuberg, C., and Hildescheimer
1911. Ueber zuckerfreie Hefegärungen, I. Biochem. Zeitsch., Bd. 31, S. 170-176.

Neuberg, C., and Kerb, J.
1913. Ueber zuckerfreie Hefegärungen, XIII. Zur Frage der Aldehydebildung bei der Garung von Hexosen sowie bei der sog. Selbstgarung Biochem. Zeitsch., Bd., 58, S. 158-170.

Neuberg, C.
1922. Von der Chemie der Gärungs-Erscheinungen. Ber. d. d. chem. Gesell., Bd. 55, S. 3624-3638.

Van Slyke, L. L., and Baker, J. C.
1918. Free lactic acid in sour milk. Jour. Biol. Chem., Vol. 35, pp. 147-178.

CORRELATION OF TEST WEIGHT PER BUSHEL OF HARD SPRING WHEAT WITH FLOUR YIELD AND OTHER FACTORS OF QUALITY[1]

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The test weight per bushel has been used as a factor of wheat quality for years. The grading systems used for wheat by the several states and boards of trade, prior to the establishment of the present federal standards, were based largely on test weight per bushel. The uniform federal standards which were established under the United States Grain Standards Act also use the test weight per bushel as a grading factor, and it is the most important grading factor in the federal standards.

The continued use of the test weight per bushel as a grading factor over a period of years, would imply that it reflects to some extent the milling value of wheat. It is generally agreed that heavy, plump wheat will usually yield more flour than shriveled wheat.

Thomas (L. M. Thomas, Bulletin 557, U. S. Dept. of Agr.) shows that the percentage of flour yielded by wheat bears a direct relation to the test weight per bushel.

Sanderson (T. Sanderson, Bulletin 137, No. Dak. Exp. Station) found that the yield of flour usually followed the grade and test weight per bushel, but individual samples showed exceptions to the rule. Bailey (C. H. Bailey, Bulletin 23, Minnesota State Department of Agriculture) made milling tests on car lots of wheat of different grades and shows that the flour yield followed the grade and test weight.

The relation of test weight to flour yield appears to be generally conceded, but very little information is available on the relation of test weight to other factors. This paper will consider, in addition to the yield of flour, the following points of flour quality: (a) color, (b) loaf volume or strength, (c) water absorbing power of flour.

Experimental

Milling and baking tests often show quite large variations between individual samples of very similar character, and these variations make the interpretation of milling and baking data a difficult task. By working out the mathematical correlation between different factors, it is possible to obtain a single expression for a number of individual samples and the correlation method has been used in this study.

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In making correlation studies, data have been used from the crop years 1919 to 1924, inclusive. Data from the 1916 crop have also been included, as a large portion of the samples handled from this crop were very low in test weight per bushel. The data for each crop have in all cases been handled separately, as this method brings out differences between crops.

Test weight per bushel is in all cases the figure obtained after grain is cleaned and scoured ready for milling. This test weight figure is somewhat higher than the test weight taken on "dockage free" wheat, but is more reliable, as it represents more nearly the weight of the wheat alone.

Yield of flour is the percentage of straight grade flour obtained—based on a given weight of wheat after cleaning and scouring and before tempering.

Color score is the mark assigned to the bread baked from the straight flour. The score assigned is a matter of judgment, but the figure can be assumed to be comparative for *any one crop year.*

Loaf volume is the figure obtained by measuring by seed displacement the cubical contents of a loaf of bread baked from 340 grams of straight flour.

Absorption is the percentage of water required to make a dough of proper consistency.

Test Weight vs. Yield of Flour

The results of correlation studies on the relation of test weight to flour yield are given in Table I. Only a summary of correlations is given, as distribution tables do not give additional information.

TABLE I

CORRELATION OF TEST WEIGHT PER BUSHEL AND YIELD OF STRAIGHT FLOUR

Data from Seven Crops of Hard Red Spring Wheat

Year	No. of sample	Test wt. per bushel—lbs. Maximum	Test wt. per bushel—lbs. Minimum	Test wt. per bushel—lbs. Average	Flour yield per cent Maximum	Flour yield per cent Minimum	Flour yield per cent Average	Coefficient of correlation	Probable error
1916	236	63.5	40.0	50.7	73.66	47.55	61.08	+.720	±0.031
1919	184	64.0	41.5	56.6	77.60	50.69	68.84	+.800	±0.027
1920	293	65.0	47.5	58.2	77.93	57.06	69.98	+.822	±0.019
1921	193	62.0	45.0	58.0	77.14	56.34	71.09	+.716	±0.035
1922	174	65.0	48.0	61.4	81.68	61.22	74.48	+.686	±0.040
1923	221	64.0	48.0	59.5	78.72	62.86	72.62	+.824	±0.022
1924	305	67.0	45.0	64.0	82.80	57.80	75.74	+.768	±0.024

Test weight determined after cleaning and scouring wheat for milling.

A high positive correlation is found in each crop year between test weight and flour yield and in all cases the coefficient of correlation is much greater than the probable error. The high degree of correlation is remarkable when we consider that the data presented cover very

different types of crops. The samples from the 1916 crop averaged only 50.7 pounds in test weight per bushel, but the coefficient of correlation for 1916 does not differ greatly from that of 1924, when the average test weight of samples was 64 pounds per bushel. The lowest coefficient of correlation is found in 1922 and the highest in 1923, but there appears to be no relation between type of crop and degree of correlation.

The results indicate that there is a high positive correlation between test weight and flour yield, and test weight is a factor of real value in wheat as it indicates the relative yield of valuable product obtainable.

Test Weight vs. Color Score of Bread

Table II gives a summary of results of correlation on relation of test weight and color. A positive correlation is found in each crop year, but the coefficient is lower and shows much greater variation than in the case of test weight and flour yield. The coefficient is in all cases greater than the probable error, but in no case do we find a coefficient of correlation greater than 0.5. For 1916, 1919, and 1921, the coefficient is greater than 0.3 and the greater degree of correlation is found in the crops which averaged lowest in test weight. The lowest coefficient of correlation is found in 1924—the year of highest average test weight.

The data indicate that a relation exists between test weight and color of flour or bread, but the correlation is not as high as the correlation between test weight and flour yield.

TABLE II

Correlation of Test Weight per Bushel and Color Score of Bread Baked from Straight Flour

Data from Seven Crops of Hard Red Spring Wheat

Year	No. of sample	Test wt. per bushel—lbs. Maximum	Minimum	Average	Color score of bread Maximum	Minimum	Average	Coefficient of correlation	Probable error
1916	236	63.5	40.0	50.7	98	30	84.2	+.407	±0.054
1919	184	64.0	41.5	56.6	101	70	90.7	+.350	±0.065
1920	293	65.0	47.5	58.2	101	50	90.0	+.283	±0.054
1921	192	62.0	45.0	58.0	95	50	89.0	+.438	±0.059
1922	174	65.0	48.0	61.4	96	50	91.0	+.277	±0.070
1923	221	64.0	48.0	59.5	95	76	91.0	+.271	±0.063
1924	305	67.0	45.0	64.0	97	50	91.9	+.173	±0.056

Test weight determined after cleaning and scouring wheat for milling.

Test Weight vs. Loaf Volume

Loaf volume is the best known index of flour strength. Table III gives a summary of correlation studies on this point. In four of the

seven years shown the probable error exceeds the coefficient of correlation, and in the other three years the correlation is not significant. A negative correlation is shown in 1916.

Summarizing the data in Table III, it appears that no correlation exists between loaf volume and test weight per bushel. If we regard protein content of wheat as an important factor in determining flour strength or loaf volume, a correlation between test weight and loaf volume would not be expected. The writers in a previous paper (Mangels, C. E. and Sanderson, T., Cereal Chemistry, Vol. II, No. 2, page 107), and also Bailey and Hendel (Bailey, C. H., and Hendel, J., Jour. Am. Society of Agronomy, Vol. 15, pp. 345-50, 1923) have shown that no correlation is found between test weight and protein content. The absence of correlation between test weight and loaf volume, therefore, is consistent with relation of test weight to protein content of wheat.

TABLE III

Correlation of Test Weight per Bushel and Loaf Volume of Bread Baked from Straight Flour

Data from Seven Crops of Hard Red Spring Wheat

Year	No. of sample	Test wt. per bushel—lbs. Maximum	Minimum	Average	Loaf volume, cc. Maximum	Minimum	Average	Coefficient of correlation	Probable error
1916........	236	63.5	40.0	50.7	3,245	1,110	2,430	—0.167	±0.063
1919........	184	64.0	41.5	56.6	2,737	1,675	2,277	+0.001	±0.074
1920........	293	65.0	47.5	58.2	2,805	1,580	2,247	+0.052	±0.058
1921........	192	62.0	45.0	58.0	2,698	1,853	2,349	+0.054	±0.072
1922........	174	65.0	48.0	61.4	2,900	1,565	2,320	+0.138	±0.074
1923........	217	64.0	48.0	59.5	2,930	1,732	2,352	+0.176	±0.066
1924........	305	67.0	45.0	64.0	3,008	1,585	2,149	+0.039	±0.057

Test weight determined after cleaning and scouring wheat for milling.

Test Weight and Absorption

Water absorption of flour is important from the bakers' viewpoint, and high grade hard wheat flours usually have a high absorption. Table IV gives a summary of correlations on relation of test weight to water absorption of flour.

TABLE IV

Correlation of Test Weight per Bushel and Water Absorbing Power of Straight Flour

Data from Seven Crops of Hard Red Spring Wheat

Year	No. of sample	Test wt. per bushel—lbs. Maximum	Minimum	Average	Absorption per cent Maximum	Minimum	Average	Coefficient of correlation	Probable error
1916..........	236	63.5	40.0	50.7	67.1	52.9	59.1	+0.024	±0.075
1919..........	184	64.0	41.5	56.6	64.1	51.8	56.2	—0.024	±0.074
1920..........	293	65.0	47.5	58.2	63.2	50.8	55.6	+0.196	±0.056
1921..........	192	62.0	45.0	58.0	61.8	51.2	57.0	+0.455	±0.057
1922..........	174	65.0	48.0	61.4	62.7	50.9	58.3	+0.092	±0.075
1923..........	217	64.0	48.0	59.5	62.3	52.6	59.0	+0.123	±0.067
1924..........	305	67.0	45.0	64.0	63.2	50.0	57.7	—0.123	±0.056

Test weight per bushel determined after cleaning and scouring wheat for milling.

The only significant correlation coefficient is for 1921, all others are too small to be regarded as significant. Five coefficients are positive and two are negative. The coefficient is less than the probable error in two cases and a coefficient three times greater than the probable error is found only in 1920 and 1921.

The coefficient varies in size and magnitude in different crop years and there is apparently no consistent relation between test weight and absorption.

Discussion

The use of the test weight per bushel as a grading factor is justified by the relation of test weight to flour yield and to a lesser extent by the relation of test weight to color of flour or bread. Wheat of high test weight will on the average produce a relatively larger amount of flour than light or shriveled wheat, and the miller is justified in paying a higher price for heavy wheat. Test weight of wheat evidently bears no relation to flour strength and this is now generally recognized by practical millers.

Summary

1. Test weight and flour yield show a high positive correlation.

2. Test weight and color score of bread show a positive correlation, but the coefficient varies with different crops.

3. No significant correlation is found between test weight and loaf volume.

4. No significant correlation is found between test weight and water absorption of flour.

5. Test weight per bushel is a real factor of value as it indicates the amount of valuable product obtainable.

COMMENTS ON THE USE OF CALCIUM ACID PHOSPHATE AS AN IMPROVER FOR SOFT WHEAT BISCUIT FLOUR

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In the course of the last decade much progress has been made in the development of our knowledge of wheat flours and their baking properties. Along with this better understanding of baking procedure has come an increasing use of certain chemicals, whose action on the flour or on the dough is such that through their use baked goods of improved appearance and eating qualities can be obtained. Many flours are so constituted that they will not yield products of the desired character with the ordinary handling; and where such flours cannot be built up through proper blending, they are the cause of trouble for both producer and consumer. If, however, the weakness of an unbalanced flour can be overcome by the addition to the flour of a small quantity of harmless gas or other chemical, all those interested in the flour are satisfied.

Opposition has developed from time to time, to the use of flour improving agents, because it was said that they helped the miller to cover up flour inferiorities, while he merchandized the improved flour in the guise of a better grade. More intelligent consideration of the matter has developed, however, the fact that such practices would not be adopted by reputable firms even though possible; while the fact that improvers often make the use of cheaper flours more acceptable to those people who cannot afford to pay for the better grades, would seem to lend economic justification to such improvement. At any rate we are growing accustomed to the benefits of flour improvement, and the values of the many types of improvers now on the market are recognized.

Skilled investigators have worked out the properties of most flour improving agents, so that they may be used scientifically; but the improver to be discussed, calcium acid phosphate, is as yet little understood, and requires further attention.

Calcium acid phosphate, or as it is commonly called, monocalcium phosphate, is merchandized as a fine, white, crystalline powder which is very soluble in water. This acid salt has a pH value of 4.0, and is commonly used as the acid ingredient of leavening mixtures because of its strongly acid reaction. Commercially, the salt is produced by treating phosphoric acid with calcium compounds, under the proper

conditions. Some years ago calcium acid phosphate was thought to be very hygroscopic; but the work of the Austrian chemist, Stoklasa, disproved this and showed the hygroscopic quality to be governed by the amount of uncombined phosphoric acid in the salt. Modern phosphate manufacturers are careful to remove this undesirable impurity, and in place of the 6 to 8 per cent of free acid which phosphate contained at the time of Jago's experiments, we now find one per cent or less in the better grades. With a free acid content running so low there is not likely to be serious trouble due to the hygroscopic nature of any high grade phosphate.

The value of an acid phosphate, for utilization in a leavening reaction, is governed by its neutralizing strength in terms of units of sodium bicarbonate, its content of free phosphoric acid, its freedom from other impurities, and its texture and color. The total neutralizing strength (theoretically) of C. P. calcium acid phosphate, is 88.8 in units of soda; but the commercial phosphates will generally average between 75 and 82 in this measurement. Trouble has frequently been experienced because of the use of several methods for the determination of phosphate neutralizing strength, for each method gives a distinctive result, and the results from the different methods do not check. One method is the so-called "cold test," which gives the lowest neutralizing strength. Another method calls for conducting the neutralizing titration at a boiling temperature, and this gives the highest result, usually from five to ten units over the results of the "cold test." The other test, and the one most nearly approximating the conditions under which the phosphate will react in practical use, employs a combination of the cold and boiling titrations, and gives results between those of the other two tests.

Although these tests are still in most general use, other and more scientific methods are being worked out for determining phosphate neutralizing strength; and it is hoped that a satisfactory method will be developed soon, and that it can be universally adopted. Under present conditions, statements as to phosphate strength may prove confusing to the non-scientific miller. There is the possibility that, on substituting in his standard self-rising formula a new phosphate, ostensibly of the same strength as the phosphate he had been using, he may cause himself serious trouble through an unbalanced leavening reaction. This could happen if the strengths of the old and the new phosphates were determined, respectively, by the "cold" and the "boiling" tests now in use.

It is believed that those flour men who are most interested in the quality of their products will endeavor to secure phosphates of the

better class, both for self-rising formulas and for use as plain flour improvers. The following analysis represents the composition and properties of a high grade phosphate, such as should be used in flour mills. This is the same phosphate as that used in our experiments.

Free phosphoric acid, per cent	1.00
Calcium acid phosphate, per cent	98.65
Calcium sulfate, per cent	.58
Phosphates of iron and aluminum, per cent	.50
Moisture, per cent	.27
Total P_2O_5, per cent	55.10
Neutralizing strength in units of bicarbonate of soda per 100 parts of phosphate:	
A	75.9
B	80.1
C	82.0

Under neutralizing strength, "A" test is the "Cold" method and "C" test is by boiling.

Fine granulation is desirable in a phosphate, as it makes possible a better distribution of this salt in the flour. Phosphates containing much coarse material are likely to run high in impurities. Color is not so important, for such small quantities of phosphate are present in phosphated and in self-rising flours, that the phosphate color will not make a noticeable difference in that of the mixture unless the phosphate is extremely dark. Phosphate color can be improved by bleaching although in some cases it grows dark again after a period of storage. The color of a phosphate is no indication of the freedom from impurities; but there can be no doubt that a phosphate of light color, and of fine, even granulation, free from specks, has been carefully made from good materials.

Little study has been given, apparently, to the effects of calcium acid phosphate as an improver for flours. This conclusion is drawn from consideration of the small amount of literature on the subject. Jago has published his investigation of the use of phosphated flours in the manufacture of yeast-leavened bread. It is generally admitted that calcium acid phosphate is a yeast stimulant, and that it improves the bread-making qualities of weak flours. Most of the literature dealing with calcium acid phosphate treats of the original type of the salt, with a high content of free acid, and possessing low strength as judged by present standards. As stated above, most of the undesirable features of the old type phosphate have been overcome through improved methods of manufacture, and it is now much more dependable, from

the flour improvement standpoint, than it was at the time of many of the experiments conducted by Jago and others, years ago.

Indications are that the first use of calcium acid phosphate as a flour improver was in England, toward the close of the last century. The English flours, due to the humidity of the atmosphere in England, and the high moisture content of the wheats, are usually soft and weak, with a tendency toward stickiness in the dough. Experiments showed that a small amount of acid phosphate, added to these flours, resulted in a firmer, more easily worked doughs, with increased capacity for absorbing water. Thus phosphate became known as a strengthener; for English bakers are accustomed to judge flour strength largely from the toughness of the dough it produces. Another result of adding acid phosphate was the speeding of the rate of dough fermentation and the stimulation of the gas production. The phosphate serves as food for the yeast plant, which accounts for its speedy growth in the dough of a phosphated flour. It was found that, with suitable allowance for the shortened fermentation period, bread with greater volume, and with improved texture and color of crumb could be produced from flours containing a small amount of calcium acid phosphate.

In the United States there has not been much use of calcium acid phosphate as an improver for flours used in making yeast-leavened bread, but there is an increasing use of this improver in flours for the biscuit trade. A few years ago economic conditions were such that soft flours from the Pacific Coast could be merchandised profitably in the southeastern states. The Southeast has always been a heavy consumer of flours which are suitable for the preparation of light breads and biscuits; but at first the soft Pacific Coast flours did not meet with much success. For the production of good biscuits by the southern methods, a flour should possess medium strength, and should yield a soft, elastic, smooth dough which is not sticky. The club wheat flour from the West did not have these properties in the right degree when used plain, but it was found, as in the case of the soft English flours, that a small amount of added phosphate would overcome, to a marked extent, the weaknesses of the flour dough. Experiments by flour millers and flour blenders, trading with the South, later showed that added phosphate would improve the baking qualities of lower grade and weaker flours from other types of wheat; and the phosphating of clear flours became increasingly common. Then the trade discovered the superior baking properties of the phosphated flours as compared with the same grades without additional phosphate; and at the present time it is almost impossible to sell flours in

some districts in the South unless the sack is marked "PHOSPHATE ADDED." The practice of phosphating biscuit flour has now spread until practically every mill catering to the soft wheat trade of the South is phosphating more or less of its flour. In many cases the higher grades, as well as the lower, are being treated with calcium acid phosphate.

In some instances flour complaints have been traced to the use of phosphate as an improving agent, but in view of the general lack of knowledge of the action of the extra phosphate in the flour and in the dough, it is a wonder that much more trouble has not been had with this improver. In most cases the flour man is aware of beneficial results from the use of extra phosphate in his flour, but he does not know the limitations of the practice, and often adds phosphate where its presence in flour is unnecessary or even a drawback. There are likewise many conflicting statements being made, principally with regard to the effect of phosphates on the keeping quality of flour and it is the intention of this paper to give some of our experience with phosphated flours, and to describe a few tests which we have made on such flours, both with respect to keeping quality, and as to physico-chemical properties.

Our first experiments with phosphated flours dealt with keeping quality. Samples of short patent and clear flour were selected, both unbleached, and with a fairly strong bleach, and made from soft red winter wheat. These flours, four in all, were each divided into four parts; and into one portion of each flour was incorporated .5% of calcium acid phosphate. Into the other three portions of each flour were incorporated, respectively, 1.0% and 1.5% acid phosphate, and a standard leavening formula. Portions of the plain flours were set aside as controls. These test flours were well sifted, and were placed in open, wide-mouthed bottles in a sheltered place exposed to the average atmospheric conditions of the St. Louis district. Heavy paper was laid over the test bottles to exclude dust and light.

Insufficient time was afforded to duplicate all possible conditions of storage, but we recognized that even the best flours, treated or untreated, will spoil in damp, hot, or dirty warehouses. We decided to conduct our storage tests on sound floors and under reasonable storage conditions. Our experience has been that most of the flour shipped to the South for domestic use has reached the ultimate consumer by the time it has been away from the mill for three months, and to make our storage experiment representative we decided to adopt this period for our test.

The test flours were examined after three months, and were found to contain an average of 11.50% of moisture, which is normal for stored flour in this climate. The self-rising flours retained their rising qualities in full. In the case of the bleached flours containing, respectively 1.0% and 1.5% of added phosphate, a slight disagreeable odor was noted. The unbleached flours containing 1.5% of added phosphate had a slight odor. All of the other samples were sweet.

To check up the three months' test we secured samples of plain and of phosphated flours from our car files. These samples, including both plain flours and similar grades treated with .5% added calcium acid phosphate, had been placed in friction-top tin cans, at a moisture content of from 12.00% to 13.00%, and had been stored between three and six months. We found, on comparing these samples, that storage had not affected the odor nor the baking quality of either the plain or the phosphated flours, and the phosphated flours exhibited the usual improved biscuit quality when baked. Self-rising flours, when stored over a long period in this manner, retained their baking strength, but exhibited a slight odor which did not appear in the biscuits baked from them.

The odor referred to in these tests is what is usually called a "warehouse odor." Some flour inspectors use it as an indication of unsoundness. This odor partakes of rancidity, although we do not believe that it results entirely from rancid fats. True rancidity has been shown to be cause principally by exposure of unsaturated fatty compounds to light and oxidation, and the reaction of the flour would not seem to have an effect. Possibly this odor results from some catalytic action of the added materials.

Our tests appeared to indicate that sound flours, under normal conditions of storage, will not deteriorate in a reasonable time if treated with not over 1.0% of calcium acid phosphate of good quality. It was noticed that unbleached flours withstood, through storage, the action of somewhat more added phosphate than bleached flours could safely carry.

Having determined that our test flours would not carry much over .5% of added phosphate through a protracted storage period, we resolved to limit our baking tests to samples containing 1.0% or less of phosphate. The following biscuit formula is our standard, and was used in all of our tests:

Leavening Mixture
(75% Formula)

Flour	227.00 grams
Phosphate	4.65 grams
Soda	3.50 grams
Salt	4.00 grams
Lard	1½ tablespoons

Skimmed milk, to make a dough of the proper consistency

Biscuit baking tests, under carefully standardized conditions, indicated considerable improvement in baking qualities of the flours to which .5% of phosphate had been added, over the qualities of similar flours, untreated. Dough quality, biscuit volume, and crumb texture and color were all improved by treating with .5% calcium acid phosphate. There was further improvement in flours with 1.0% added phosphate over those with .5% although the improvement was principally in crumb color. In the case of the flour with 1.0% phosphate the dough was very soft, though not sticky. The absorption increased gradually through the plain flour, the flour with .5% phosphate added, and that with 1.0% phosphate added.

In deference to the sour milk biscuit baker of the South we made a comparative test, using lactic acid in place of the added phosphate. We discovered that by using as much as one cubic centimeter of 93% lactic acid we could make better biscuits than we could from the same flour containing added phosphate instead. The lactic acid dough was quite soft and elastic, as would be expected; but it was determined that a small excess of lactic acid would make the dough too soft to handle. The parallel results obtained by the use of lactic acid and acid phosphates led us to believe that most of the improving effect of the acid phosphate was due to manner in which it increased the acidity of the dough. There was another effect, however, for although the same general dough and biscuit improvement was obtained with the phosphate as with the lactic acid, the latter did not increase the absorption of the dough as did the phosphate, and the phosphated doughs were tougher and more easily worked.

We were interested in determining the reason for the marked improvement in crumb color, which was secured by adding either phosphate or lactic acid. Increased lightness and improved texture of crumb did not seem to account for all of the improvement noted. The gluten was washed out of doughs made from plain patent and clear flours, and from the same flours containing respectively .5% phosphate, 1.0%

phosphate, and 1 cc. of lactic acid. It was found, incidentally, that the phosphate and lactic acid made the glutens hard to collect; the dough with 1.0% phosphate working very soft, and the lactic acid dough producing a "runny" gluten. All glutens were of the same color, showing that there was no bleaching of the gluten through added acid compounds. It then occurred to us that we had noticed, in other work, that flour contains an indicator material which is de-colorized at a certain acidity, while appearing brownish at lower hydrogen-ion concentrations. It seems likely that this indicator material carries part of the secret of the improved color which is noticeable in doughs and biscuits from acidified flours.

Samples of our soft wheat patent, our soft wheat clear flour, and of a club wheat straight which came to hand, were each divided into two portions. One portion of each was thoroughly blended with .5% calcium acid phosphate, and the other portion was untreated. These were bleached flours. The samples thus obtained were then tested for hydrogen-ion concentration, buffer value, viscosity, and soluble protein. The pH and buffer values were obtained after the manner recommended by Weaver and Goldtrap (1922). The viscosity was determined after washing out the electrolytes as recommended by Gortner (1924). The results of these tests were as follows:—

Sample	pH of flour	pH of Buffered flour	Buffer value	Soluble protein, per cent	Viscosity, W. & T.
Soft patent....................	5.985	4.953	10.32	2.33	222
Soft patent, 5% phosphate...	5.478	4.936	5.42	3.05	183
Soft clear.....................	6.069	5.359	7.10	1.86	256
Soft clear, 5% phosphate....	5.647	5.173	4.74	2.68	217
Club straight.................	5.816	5.139	6.77	2.08	125
Club straight, 5% phosphate	5.511	4.987	5.24	2.88	81

These tests show that the presence of added calcium acid phosphate in flour doughs tends to increase the hydrogen-ion concentration and buffer values of the doughs, and as would be expected, the soluble proteins are increased in amount. A decrease in viscosity is noted, which may or may not be due, to failure to remove, by leaching with distilled water, all of the electrolyte in the phosphated flours.

Smith and Bailey (1923), published some interesting work on the effects of baking powder residual salts on doughs and on biscuits, and in connection with our work, their findings make some interesting deductions possible. They have shown that: "Gluten properties, including elasticity and hydration capacity, are affected by baking powder, and by the residual salts resulting from the chemical reaction of baking powder ingredients." They showed that the effect of all baking powders was such that the hydrogen-ion concentration of the doughs

and of the resulting biscuits was reduced, this reduction being more marked in the case of phosphate baking powders than with cream of tartar or S. A. S. powders. They indicate that phosphate baking powder residues contain disodium phosphate and tricalcium phosphate in considerable amount, and that these two very alkaline salts reduce the effective acidity of the medium, while the sodium salts present in other types of baking powder residues are not so strongly alkaline as those from the phosphate powders.

We know that the gluten proteins tend to imbibe water, and disperse in a weakly alkaline medium, so that a slightly alkaline dough is sticky. In making biscuits, the preparation of a dough of the right character is essential for the best results, and a soft, smooth elastic dough cannot be secured from flour tending to become sticky when mixed with the usual amount of liquid. An excess of alkali in the dough is also marked by a yellowish color and by a "soapy" taste. In all of our baking tests with biscuits we have found that a slight excess of acid material, such as phosphate, which would reduce the alkaline tendency of the leavening charge, would likewise make for improved quality of dough and of finished product. We have found that even a slight excess of alkali is harmful, while 1% of acid phosphate can be used without bad results. In view of the increasing popularity of flours acidified with phosphate and considering the fact that most baking powders have their acid and alkaline ingredients closely balanced, it would seem that more strongly acid baking powders could be used, particularly of the phosphate type. Fairly strong (acid) leavening formulas for self-rising flours are much less to be feared than those containing a larger proportion of sodium bicarbonate.

Clear flours and other lower grades, which have a lower hydrogen-ion concentration, require more safeguards against alkalinity of dough than the higher grade flours, and flours very lightly buffered, as are some club wheat flours, would seem to require some help in retaining their acidity in the baking powder dough. Experiments show that flours of these types do benefit the most from treatment with acid phosphate. It is thought that the acidifying effect which added phosphate has on flour doughs is more important than any salt effect which it may have, although the salt effect has some significance.

In conclusion it might be well to touch on the manner in which the phosphate is corporated in the mixture with flour. Most of those who phosphate flour use either a batch mixer, or a feeder which adds the salt directly to the flour stream. The batch mixer is by far the more reliable of the two, provided that care is used in adding the correct charge of phosphate, and that the period of mixing is suffi-

ciently long. It should be borne in mind that the longer the mixing is carried on, the better will be the results; and that a short mixing is dangerous. Phosphate feeders, for various reasons, may cause an extreme variation in the proportion of phosphate added to the flour stream, and as much as 10% added phosphate in a flour served by a phosphate feeder has been observed. You can imagine the effect of 10% added phosphate on a flour, if 1% has been determined as the limit for improving purposes. It should be understood that phosphate, when present in too great concentration, has a distinct effect on flour keeping qualities, particularly in the case of flours with fairly strong bleach, such as are used by the soft wheat trade of the South, and more care should be used in securing proper distribution of the phosphate in the flour.

Much benefit is to be obtained from the use of calcium acid phosphate as a flour improver, if the higher grade of the salt is used, if flour characteristics are studied and their response to the added phosphate is noted, and if care is used in blending the acid phosphate with the flour. There is still a great deal of work to be done before the full effect of calcium acid phosphate upon flour is explained, but it is hoped that this contribution may be of some value in shedding light on the subject.

In concluding we wish to record our thanks to Roland J. Clark for his co-operation in determining the hydrogen-ion concentration of our preparations.

Literature Cited

Smith, Florence C., and Bailey, C. H.
1923. The effect of chemical leavening agents on the properties of bread. Jour. Amer. Ass'n Cereal Chemists, Vol. 8, pp. 183-194.

Gortner, R. A.
1924. Viscosity as a measure of gluten quality. Cereal Chem., Vol. I, pp. 75-81.

Jago, Wm., and Jago, Wm. C.
1911. The technology of bread-making.

A STUDY OF THE DETERMINATION OF THE NEUTRALIZING VALUE OF MONOCALCIUM PHOSPHATE

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The determination of the neutralizing value of monocalcium phosphate has for some time been a much discussed problem. In view of the fact that this baking acid has been in use over a considerable period of time and is giving satisfactory results, it seems strange that the method for determining the concentration which should be used is still in question. The situation, therefore, warrants some elucidation. There are available as baking acids, several different grades of monocalcium phosphate. Owing to differences in raw materials and in method of manufacture, these phosphates have different strengths; i.e., in the baking operation the several available phosphates will react with different amounts of sodium bicarbonate. Thus, 100 pounds of one phosphate will require 75 pounds of bicarbonate, whereas, an equal weight of another will need 80 pounds for its complete utilization as a leavening agent. In the terms usually employed, the former phosphate should be used at a strength of 75%, the latter at a strength of 80%. It is because of this variation in strength of the different monocalcium phosphates obtainable, that it has been desired to obtain a laboratory test by which the reacting value of these phosphates could be predetermined.

The method of analysis, which has been the object of much work in recent years, must meet rather rigid requirements. The results obtained in the laboratory must be readily translatable into baking practice and be in accord with it. The confusion which exists at the present time in connection with the neutralizing value of phosphates can be traced partly to the fact that the reactions between monocalcium phosphate and bicarbonate of soda are complex and partly to the difficulty of even approximately duplicating, in any laboratory test, the conditions obtaining in the oven. In view of these conditions, it may be indeed difficult to develop a laboratory test, preferably simple, to account for these involved reactions. It seems that the most that can be reasonably expected is that the method be fundamentally valid and that it give results comparable to those obtained in practice, even though the methods of carrying out the reactions in the laboratory be totally different from the way the reactions take place in the oven. With this point of view in mind it may be worth while to examine what has been done by way of establishing a laboratory method for the determination of the neutralizing value of monocalcium phosphate.

It may not be amiss first to note the work which has been done in the study of the complex reactions taking place during neutralization of phosphoric acid by different bases. Wendt and Clarke (1923) call attention to the fact that when phosphoric acid is titrated electrometrically using lime-water as the base, rearrangements, notably of dicalcium phosphate to give mono and tricalcium phosphate, occur which cause the accurate titration to be difficult. The conclusion drawn from the experiments reported is that the neutralization of monocalcium phosphate in baking ends somewhat before it is completely changed to tricalcium phosphate. Similar results were reported by Davis and Maveety (1922) in experiments performed to determine whether di- or tricalcium phosphate was formed exclusively in solutions not markedly alkaline. It was found that a mixture of the two was obtained, which would indicate that the neutralization did not go completely to the tricalcium stage.

More recent work by Holt and La Mer (1925a, 1925b), and by Hoffman and Gortner (1925) has confirmed this conclusion, and at the same time presented different mechanisms for the reactions involved in the neutralization of phosphoric acid by calcium hydroxide. According to Holt and La Mer, during the course of the titration dicalcium phosphate precipitates, then redissolves, the less soluble tricalcium phosphate being thrown out of solution. The result is that di- and tricalcium phosphates are formed simultaneously, and the neutralization curve shows a break only after both the secondary and tertiary hydrogens of the phosphoric acid have been neutralized. Hoffman and Gortner (1925) also present data showing that tricalcium phosphate is precipitated at the same time as dicalcium in the course of the neutralization, and that it is stable in solutions having a pH of 6.5. The reactions involved are accounted for on the basis of the structure of the dicalcium phosphate molecule. For a complete discussion, the reader is referred to the original paper.

In none of the work reported has a complete study been made of the neutralization of monocalcium phosphate with bicarbonate of soda, where additional complications arise due to the presence of the two bases. The equations supposed to represent the possible reactions in this neutralization have been frequently published. For convenience, we are repeating them here:

(1) $3CaH_4(PO_4)_2.H_2O+8NaHCO_3=Ca_3(PO_4)_2+4Na_2HPO_4+8CO_2+11H_2O.$

(2) $3CaH_4(PO_4)_2.H_2O+6NaHCO_3=3CaHPO_4+3Na_2HPO_4+6CO_2+9H_2O.$

(3) $3CaHPO_4+2NaHCO_3=Ca_3(PO_4)_2+Na_2HPO_4+2CO_2+2H_2O.$

In the light of the work of Hoffman and Gortner (1925), it must be recognized that these reactions occur simultaneously, and that these

simple equations do not give an accurate picture of the mechanism of the reactions. They are useful, however, in making calculations and for that reason have been included.

The Direct Titration of Monocalcium Phosphate

All of the analytical methods proposed for the determination of the neutralizing value of this acid have been essentially titration methods although they have differed considerably in the manner in which they were to be carried out in the laboratory. Thus the tentative method of the Association of Official Agricultural Chemists (1925) was a direct titration method. That proposed later by Bailey (1925a) in reality called for the determination of points on the titration curve by obtaining the hydrogen-ion concentration of different mixtures of monocalcium phosphate and bicarbonate with a view to finding the mixture having a neutral residue. The method also postulates that at this point there will remain no undecomposed bicarbonate, and that for pH greater than 7.0 there will be undecomposed bicarbonate, as indicated by the presence of CO_2 in the residue.

The A. O. A. C. tentative method called for the determination of the neutralizing value according to the following procedure:

> Weigh 0.84 grams of mono-calcium phosphate into a small beaker or casserole, add 25cc. of water and 5 drops of a 0.2 per cent solution of phenolphthalein, and titrate with 0.2 N sodium hydroxide to a faint pink; then heat to boiling; boil 1 minute; and continue the titration, while the solution is hot, to a faint pink color again. (Add the bulk of the standard alkali solution rapidly and with vigorous stirring.) The total buret reading multiplied by 2 equals the neutralizing strength of 100 parts of phosphate in terms of sodium bicarbonate.

This method was submitted to parties interested in the determination and collaborative results obtained. These results have recently been published by Bailey (1925b). The collaborators in general agreed quite satisfactorily. No action was taken on the method, however, inasmuch as it was thought desirable to obtain results on the method suggested by Bailey (1925a) to which reference has been made and which will be discussed later.

There are several minor criticisms which might be made of the way in which the A. O. A. C. tentative method is carried out. It has been found for instance, that in order to obtain concordant results in phosphate titrations it is necessary to follow precisely the same procedure every time. The method under discussion does not indicate the length of time to be taken to get the solution to boiling, and it has been the experience of this laboratory that varying this time will give discordant results. This is possibly due to the fact that it requires time for equilibrium to be established as was indicated in the work of Wendt and Clarke to which reference has already been made. The

chief criticism of the method is that the results obtained by the use of it do not agree with baking tests. It has been found that phosphates can be used in baking at values several points higher than the neutralizing value indicated by this direct titration. Unless then, the value obtained from the titration is corrected the method does not tell the quantity of bicarbonate to use with the phosphate under examination. To the extent of specifying the quantity of indicator, the concentration of the sodium hydroxide and the time of boiling, the method might be classed as empirical. The variation of one or more of these conditions would give different results. It ought to be possible, therefore, to alter them so as to obtain a value capable of being used in baking without any correction. The method would then of course be suitable for the determination of the baking value of monocalcium phosphate. Work directed to this end was started in this laboratory several years ago and will be discussed below. It is advisable first to consider the other method proposed by Baiiey (1925).

The Hydrogen-ion Method

The method more recently proposed and hereafter referred to as the "proposed method" in contradistinction to the present tentative A. O. A. C. method, is somewhat more complex in its conception and merits detailed discussion. The basis of the method is given in the report of the collaborative work for 1924 by Bailey (1925b). It was there stated that "a neutral baking powder would result and there would be no residual carbon dioxide if just enough bicarbonate of soda were added to react with the acid of the phosphate." This amount of bicarbonate, which is the measure of the neutralizing value, was to be determined as follows:

> To 26.73 grams of sodium bicarbonate add 41.77 grams of the monocalcium phosphate to be tested. (This assumes a neutralization value of 64.) Also add if desired, 31.50 grams of neutral starch and mix thoroughly. Keep dry until used. Add 1 gram of this test baking powder to 100 cc. of distilled water in a 250 cc. Pyrex beaker. Boil until free from carbon dioxide. When cool determine the pH of the supernatant liquid electrometrically or colorimetrically, using appropriate indicators.

Different mixtures were to be prepared in the manner just described until a mixture was obtained indicating a pH of 7.0. The ratio of the bicarbonate to the phosphate in this mixture gives the neutralizing value of the phosphate. It will be seen that the method as we have indicated above consists of the determination of individual points on the titration curve as ordinarily obtained electrometrically or colorimetrically. The neutralizing value is given by the intersection of the curve so obtained with the line corresponding to a pH of 7.0. This fact brings up the question as to whether we are not thus arbitrarily

fixing a neutralizing value without taking into consideration the substances with which we are dealing.

Everyone acknowledges the fact that a pH of 7.0 constitutes neutrality, but it does not have any significance with reference to the completion of the neutralization reaction as shown by Clark (1920). It is common knowledge that the point where neutralization is complete depends upon the acid and base involved. With a strong acid such as hydrochloric and a strong base such as sodium hydroxide, the reaction is actually complete at a pH of 7.0. In the case of a weak base and a stronger acid the reaction reaches completion on the acid side of pH = 7.0, whereas with a weak acid and a stronger base the neutralization is complete on the alkaline side of pH = 7.0. It seems doubtful then that we are justified in imposing this arbitrary limit on the neutralization of phosphate with sodium bicarbonate or with sodium carbonate, since upon boiling the bicarbonate is transformed to the normal carbonate.

The work of Wendt and Clarke, to which reference has been made, has indicated that a pH of 7.0 is very little, if any, beyond the neutralization of the phosphate to the dicalcium stage as in equation No. 2 above. If the reaction in baking stops at this point it is proper to call this the end-point of the reaction and to calculate from it the neutralizing value of the phosphate.

Neutralizing Value of Dicalcium Phosphate

In order to prove whether the neutralization of monocalcium phosphate stops at the dicalcium stage, some experiments were carried out using dicalcium phosphate as a baking acid. A number of mixtures of crystalline dicalcium phosphate, $CaHPO_4 .2H_2O$, bicarbonate of soda, and starch were prepared so as to give baking powders having approximately uniform carbon dioxide content in the neighborhood of 5.3%. These mixtures were allowed to react, the directions of the proposed method being followed and the hydrogen-ion concentrations of the residues were determined. The residual carbon dioxide was also determined using the tentative A. O. A. C. method (1925), section 12. The results of these experiments are recorded in Table I.

The results given in Table I indicate that dicalcium phosphate can be assumed to have a value of 25 and still yield a residue which is more acid than pH=7.0. This means that the reaction between monocalcium phosphate and sodium bicarbonate is not complete when all the monocalcium has been changed to dicalcium phosphate. When monocalcium phosphate is used on the basis of a value of 80 it is found that the pH of the residue is in the neighborhood of 7.8. The difference between this value and the value obtained for the mixture of dicalcium

phosphate and soda given in the above table is due to the fact that we have a higher relative concentration of disodium phosphate where monocalcium phosphate is used as the baking acid. Inasmuch as disodium phosphate has a pH in the neighborhood of 9 it is readily seen that when it is present in appreciable quantities it tends to increase the relative alkalinity of the mixture. From these experiments it would appear that fixing 7.0 as the pH for the determination of the neutralizing value of monocalcium phosphate is not warranted by the reactions involved, which do not reach completion at this point.

TABLE I

HYDROGEN-ION CONCENTRATION AND RESIDUAL CARBON DIOXIDE CONTENT OF BAKING POWDERS

Mixture	Soda-phosphate ratio	Total CO_2	pH	Residual CO_2
		Per cent		Per cent
Soda .... 10% Crystalline dicalcium 90%	11.1	5.32	4.75	0.27
Soda .... 10% Crystalline dicalcium 66.6% Starch 23.4%	15.0	5.35	5.49	0.35
Soda .... 10% Crystalline dicalcium 50% Starch 40%	20.0	5.30	6.09	0.30
Soda .... 10% Crystalline dicalcium 40% Starch 50%	25.0	5.30	6.61	0.60

Further confirmation of these results was obtained by making some baking tests using dicalcium phosphate as the baking acid. It was used at a strength of 25 and it was found that the biscuits obtained were of good color, good volume, and were satisfactory in every respect. In order to determine whether the disodium phosphate which is formed during the neutralization of monocalcium phosphate has any deleterious effects upon the biscuits, a test bake was made in which the quantity of disodium phosphate ordinarily formed in a phosphate baking powder was added to the baking powder made of dicalcium phosphate. The biscuits obtained were again entirely satisfactory The pH of the residue from this last baking powder was determined and was found to be 7.91, the residual carbon dioxide being 0.2%. In the data given below it has been indicated that when monocalcium phosphate is used on the basis of a value of 80, a pH of 7.89 is obtained and a residual carbon dioxide of 0.3%. The dicalcic baking powder, with the disodium phosphate added, behaves exactly as the monocalcium phosphate does.

Residual Carbon Dioxide

The other characteristic of the mixture of bicarbonate and phosphate representing the neutralizing value of the latter, according to

the proposed method, is the absence of residual carbon dioxide. Several series of experiments were performed to determine whether there is any relation between the pH of the baking powder residue and the residual carbon dioxide contained by it. This work was not confined to mixtures of monocalcium phosphate and bicarbonate, but other baking acids were used as well. Mixtures of the several acids and bicarbonate were made up at different strengths and allowed to react in accordance with the procedure outlined in the proposed method. The hydrogen-ion concentrations of the solutions were determined as directed and the residual carbon dioxide was obtained on each mixture. The results are given in Table II.

TABLE II

COMPARISON OF THE H-ION CONCENTRATION OF BAKING POWDER RESIDUES WITH THE PERCENTAGE OF RESIDUAL CARBON DIOXIDE

Baking acid	Strength	pH	Residual CO_2
			Per cent
Sodium aluminum sulphate	100	7.02	0.45
Sodium aluminum sulphate	90	5.58	0.15
Cream of tartar	44.7	7.28	0.00
Phosphate	80	7.89	0.30

These results given in Table II together with those obtained on the mixtures of dicalcium phosphate and bicarbonate give values over a wide pH range. The striking thing is that there is no direct relation between the residual carbon dioxide and the hydrogen-ion concentration of the baking powder residue. Thus the residue from the reaction with cream of tartar, having a pH of 7.28 contains less residual carbon dioxide than the alum residue at a pH of 5.5. In the case of the dicalcium phosphate mixture there is no appreciable increase in the residual carbon dioxide over a considerable range of hydrogen-ion concentration. It has thus been found impossible to correlate, in the laboratory, the pH of the baking powder residue with residual carbon dioxide. It is also evident that the determination of such small amounts of carbon dioxide with any degree of accuracy is too difficult to form the basis of a determination of the neutralizing value of phosphate.

During the course of the work on this problem it occurred to us that the reason for carbon dioxide being present in the residue even when the monocalcium phosphate was being used at its generally accepted baking value, might be due to the formation, during the reaction, of some calcium carbonate which, because of its slight solubility would react but very slowly if at all. In order to prove this point some further work was done on the dicalcium phosphate baking mixtures to which reference has already been made. The experiments

were carried out in the following manner: The factor weight of the baking powder as used in the Chittick carbon dioxide apparatus was allowed to react according to the usual method for the determination of residual carbon dioxide. Before the residual carbon dioxide was determined, however, the mixture was filtered and washed with distilled water. The precipitate and filtrate were tested separately for carbon dioxide. The results are shown in Table III.

TABLE III

RESIDUAL CARBON DIOXIDE AFTER THE REACTION OF DICALCIUM PHOSPHATE BAKING POWDERS OF VARYING COMPOSITION

Composition of baking powder: Dicalcium phosphate, $CaHPO_4$, $2H_2O$	Bicarbonate of soda	Starch	CO_2 content baking powder	Residual CO_2, water soluble	Residual CO_2, water insoluble
Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
90	10	0.0	5.32	0.06	0.35
66.6	10	23.4	5.35	0.00	0.45
50	10	40	5.30	0.05	0.50
40	10	50	5.30	0.05	0.25
33.3	10	56.7	5.28	0.06	0.45

It is evident from the experiments recorded in Table III that the greater part of the residual carbon dioxide present when monocalcium phosphate is neutralized beyond the dicalcium stage, and not past the point of complete neutralization, is present in an insoluble form. It is not, under these conditions, in the residue as undecomposed bicarbonate or carbonate of soda. The amount of residual carbon dioxide found when monocalcium phosphate is used at 80 is of the same order of magnitude as that obtained in these mixtures, namely 0.3%. The increased alkalinity in the latter case is undoubtedly due to the presence of disodium phosphate, and not to sodium carbonate which was not acted upon by the phosphate.

The experiments which have here been reported were undertaken to get additional knowledge of the extent of the neutralization of monocalcium phosphate by bicarbonate of soda. They have confirmed the work of previous investigators in showing that the reaction goes beyond the dicalcium stage. They have also indicated that the current notions about residual carbon dioxide are not altogether accurate in assuming it to be due entirely to undecomposed soda.

The work also demonstrates the inadvisability of accepting a method for the determination of neutralizing value, which separates so very widely this value from the strength at which the acid can be used in baking, as to make the practical application of the method impossible. L. H. Bailey, the author of the method, has no intention of indicating the strength at which the phosphate is to be used in baking,

but proposes the method only to determine the value which will produce a neutral residue. In carrying out the method in the laboratory, there are some slight difficulties encountered, possibly worthy of mention. It has been the experience of this laboratory that considerable time is required for equilibrium to be established, and unless a definite time is set, concordant results are not obtained. This would be a minor objection indeed, if the results were capable of application in baking. This is too much to expect of it, however, inasmuch as the method definitely fixes the point to which the reaction is taken, regardless of the fact that this point is not the end-point of the reaction. The method will tell when the "neutral line" is crossed, but that is all.

The Indirect Titration of Phosphates

In this laboratory the difficulty of obtaining a satisfactory solution to this problem has been recognized. It has also been realized that all methods must necessarily have limitations, because of the complexity of the reactions. The development of a method has been more with a view to applying it in baking than to get a complete solution of the entire problem. The result has been a titration method which has been in use for several years and has enabled analysts to determine the baking values of different phosphates with satisfactory precision. On the other hand, it is easy to see that the entire method is empirical, and if the conditions under which it is carried out are varied to any great extent, the results will not be in agreement. This is true, and would be true of any method for the titration of phosphates, due to properties inherent in the reacting substances and the products of the reaction. If, however, the procedure is followed with a reasonable degree of care, it will give results which can be directly translated to baking values, and when applied will yield satisfactory oven products.

The method differs from the A. O. A. C. titration method discussed above, in being indirect. This procedure was adopted because a sharper end-point is obtained than when the phosphate is titrated directly. The other directions specified were determined after considerable work to be those which give the most concordant results, as well as those most suited for application to baking. It will be noticed that only one drop (0.05 cc.) of indicator is used. The quantity of indicator plays an important part in the end-point of a phosphate reaction, as will be appreciated by those who have had much to do with the volumetric determination of phosphate. It has been therefore found necessary to fix the amount of indicator to get concordant results; similarly with the time of heating and of boiling. The method recommended is as follows:

Weigh out 0.84 gram of the phosphate and place in a casserole. Add 25 cc. of cold water and after stirring for a moment, add exactly 90.0 cc. 0.1 N sodium hydroxide. Bring the solution to a boil in two minutes and boil for one minute. While the solution is still boiling hot add 0.05 cc. of 0.25% phenolphthalein solution and then 0.2 N hydrochloric acid until the pink color of the indicator has all but disappeared. Boil the solution then for one minute and again add at once standard hydrochloric acid until the pink color has just disappeared.

Multiply the total number of cubic centimeters of standard acid used by 2 and subtract from 90.0. The difference is the baking value of 100 parts of the phosphate in terms of bicarbonate of soda.

It is not claimed that the development of this method has solved all of the problems related to the neutralization of monocalcium phosphate. The reactions remain as complex as before, and it is hoped that further study of them will continue, so that a more complete understanding of them may be gained. The titration of monocalcium phosphates by this method has, however, helped in determining the values at which they can be baked. It has been a useful tool, and as such is offered for more general use.

Summary

1. The methods at present available for the determination of the neutralizing value of monocalcium phosphate have been studied with a view to estimating their merit in calculating the proportions of phosphate and bicarbonate to be used in baking. It has been indicated that titration methods, either direct or indirect, can be so manipulated as to fulfill these requirements. The method which involves the determination of the pH of the baking powder residue does not give applicable results, and further does not take into consideration the nature of the reacting substances.

2. Experimental evidence has been advanced to show that the reaction between monocalcium phosphate and soda does not stop at a pH of 7.0, nor at the dicalcium stage. It has been shown that dicalcium phosphate can be used as a baking acid, giving satisfactory products when used at a strength of 25.

3. Within the limits of baking practice residual carbon dioxide does not indicate undecomposed soda, and is not directly related to the pH of the residue. It has also been shown that the residual carbon dioxide is present to a large extent in an insoluble form.

4. A method has been proposed which is capable of accurate manipulation and practical application in baking, and which has been in use successfully for a number of years.

Literature Cited

Association of Official Agricultural Chemists
1925. Methods, p. 306.
Bailey, L. H.
1925a. The "neutralizing value" of monocalcium phosphate. Jour. Ass'n Off. Agr. Chemists, Vol. 8, pp. 444-447.
1925b. Report on baking powder. Jour. Ass'n Off. Agr. Chemists, Vol. 8, pp. 490-495.
Clark, W. M.
1920. The determination of hydrogen ions. 2d Edition, p. 302.
Davis, C. E., and Maveety, D. J.
1922. Leavens: Their action and measurement. Jour. Ind. Eng. Chem., Vol. 14, pp. 210-212.
Hoffman, W., and Gortner, R. A.
1925. Physico-chemical studies on proteins II. Alkali binding. A comparison of the electrometric titration of proteins and of phosphoric acid with sodium and calcium hydroxides. Jour. Phys. Chem., Vol. 29, pp. 769-781.
Holt, L. E., Jr., La Mer, V. K., and Chown, H. B.
1925a. Studies in calcification I. The solubility product of secondary and tertiary calcium phosphate under various conditions. Jour. Biol. Chem., Vol. 64, pp. 509-567.
1925b. (With assistance of I. Gittelman.) Studies in calcification II. Delayed equilibrium between the calcium phosphates and its biological significance. Jour. Biol. Chem., Vol. 64, p. 567.
Wendt, G. L., and Clarke, A. H.
1923. An electrometric study of the neutralization of phosphoric acid by calcium hydroxide. Jour. Am. Chem. Soc., Vol. 45, pp. 881-887.

SOME OBSERVATIONS ON MAKING ASH DETERMINATIONS

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At the present time there seems to be no uniform method in use for making ash determinations on flour. The method of the Association of Official Agricultural Chemists is used mainly as a guide for the preparation and development of other methods. This is due, somewhat, to the elastic interpretation of the method of the association and partially to the desire of the cereal chemists for a shorter method for mill control work. Several methods for making ash determinations have therefore, been developed in which the size of sample, time of ashing, and temperature of ashing have been varied to suit the desires of the individual. The ashing of flour at dull red heat, as recommended, is unsatisfactory. Heat which may appear to be dull red on a sunny day is much less than this on a dark day. Of interest in this connection are the following figures, comparing color with actual degrees of temperature.

Color	Degrees F.	Degrees C.
First visible red	900	482
Dull red	1,175	635
Bright red	1,550	843
Orange	1,725	941
White	2,200	1,200
Dazzling white	2,732	1,500

It takes longer to ash large samples than small samples, and the rapidity of ashing is also influenced by the size of the ashing crucible. Fusion of the ash is frequently carried on when rapid results are desired. How much these variables will influence the final test is the subject of this paper.

Equipment Used

The ashing oven used was electrically heated and of the replacable unit type, having the following inside dimensions: 7½ by 5½ by 14 inches. Temperatures were controlled by an automatic electric pyrometer having a range of 25 to 1000 degrees C. in 10-degree intervals. The pyrometer was guaranteed to be accurate to within 10 degrees.

The ashing crucibles used throughout this investigation, unless otherwise stated, were of porcelain, 20 millimeters deep, 40 mm. in diameter, and of 25 mm. capacity.

The size of the sample of flour used was three grams, unless stated differently.

Straight grade flours from the five commercial classes of wheat were ashed.

Experimental

Before starting the investigation, the oven was tested for efficiency and "hot spots." Two samples of flour were used, one with a high-ash and the other with a low-ash content. After thoroughly mixing the flour, thirty-two determinations were made on each sample, so that every available place in the muffle was taken. The flours were allowed to ash over night at a temperature of 585° C. In making the test, the muffle door was closed to within an inch of the floor. There was no place for ventilation in the rear of the muffle, the usual space for ventilation being occupied by the pyrometer.

The results of the tests revealed no hot spots, but it developed that incomplete combustion takes place in the first two rows and in the last row of the furnace. In other words, to get uniform results with no ventilation in the rear of the muffle, the area one inch from the rear wall, and that three inches from the muffle door should not be used.

More efficient ventilation was then given the muffle by making a one-inch hole in the back wall. The tests were repeated as before, except that the door of the muffle was fully closed and the small mica window opened, giving a through draft. The result of this procedure was to promote more efficient oxidation and to extend the area that could be used for ashing 1½ inches nearer to the front of the muffle.

With no through ventilation, it is quite necessary to start with a hot muffle (above 450° C.) and leave the muffle door open until partial combustion has taken place, as the accumulation of gases from gradual heating will often explode, with results disastrous to the determinations.

This can be seen from a glance at Table I, in which the data are given from the same samples after an explosion took place, and when it was prevented. The average difference amounted to 0.11 per cent, with a maximum loss of 0.18, and a minimum loss of 0.06 per cent. The loss is progressive from the rear to the front of the muffle.

Effect of Temperature on the Ash Determinations

To determine the effect of temperature on the ash results, five samples of straight grade flour, representing the five commercial classes of wheat, were tested under varying conditions. Four series of tests were made with each sample. To insure uniformity of oven heat, the samples were run in quadruplicate and placed systematically throughout the oven. The precautions pointed out in the previous section were taken into consideration, in making these tests.

TABLE I

EFFECT OF AN EXPLOSION ON THE PERCENTAGE OF FLOUR ASH

Sample No.	Row in muffle*	Ash % after explosion	Ash % explosion prevented	Difference %	Av. % difference each row
11739	1	.18	.42	.24	...
40	1	.33	.45	.12	...
41	1	.37	.48	.11	...
42	1	.37	.45	.08	.14
43	2	.06	.45	.39	...
44	2	.35	.48	.13	...
45	2	.38	.47	.09	...
46	2	.42	.52	.10	.18
47	3	.38	.48	.10	...
48	3	.40	.45	.05	...
49	3	.38	.58	.20	...
50	3	.37	.50	.13	.12
51	4	.38	.42	.04	...
52	4	.37	.50	.13	...
53	4	.40	.40	.00	...
54	4	.47	.43	.04	.04
55	5	.47	.50	.03	...
56	5	.50	.53	.03	...
57	5	.33	.45	.12	...
58	5	.38	.45	.07	.06
59	6	.33	.43	.10	...
60	6	.38	.47	.09	...
61	6	.42	.50	.08	...
62	6	.28	.42	.14	.10
Total av. % difference	..	...	...	...	.11

*Front to back.

In Series 1, the samples were heated for 16 hours at 485° C., cooler, and weighed. They were replaced in the muffle and reheated to 585° C. for 16 hours longer, and again cooled, and weighed. Finally they were heated to 685° C. for 16 additional hours, cooled, and weighed, making in all 48 hours of actual heating.

In Series 2, the initial temperature of 485° C. was eliminated and the ashing accomplished in two steps, i.e., at 585° C. and 685° C.

In Series 3, the influence of fusion on the ash results was studied. The data from these studies are given in Table II.

The temperature of 485° C. was not sufficiently high completely to oxodize any of the samples of flour under test. The ash at this temperature was black to gray black. Reheating at 585° C. improved the appearance of the ash considerably, but there was still carbon in the ash of many samples. At 685° C. the ash, on the whole, was free of carbon. In some instances, however, carbon was occluded and in all cases fusion had taken place. Fusion had also started at 585° C. Although the ash was practically carbon-free at 685° C. the results were quite irregular. The most uniform results were obtained by heating the sample for 16 hours at a temperature of 585° C., higher or lower temperatures yielding irregular results.

TABLE II

EFFECT OF TEMPERATURE ON THE ASH DETERMINATIONS

Sample No.	Class	Series 1 Heated 485°C.	Series 1 Reheated 585°C.	Series 1 Reheated 685° C.*	Series 2 Heated 585° C.	Series 2 Reheated 685° C.	Series 3 Fused in hot muffle cooled in dessicator 685° C.	Series 3 Reheated to 785 C. after fusion cooled in dessicator	Series 4 Glycerine and alcohol heated 570° C.
11105	Durum	.68	.67	.63	.63	.67	.68	.67	.63
		.65	.62	.60	.63	.63	.63	.63	.63
		.67	.67	.62	.63	.63	.68	.68	.63
		.67	.67	.63	.65	.65	...	...	.64
	Average	.67	.66	.62	.63	.64	.66	.66	.63
	Maximum	.68	.67	.63	.65	.67	.68	.68	.64
	Minimum	.65	.62	.60	.63	.63	.63	.63	.63
	Max. difference	.03	.05	.03	.02	.04	.05	.05	.01
11113	Hard	.48	.47	.43	.45	.47	.52	.48	.45
	Red	.47	.45	.43	.45	.43	.45	.45	.44
	Spring	.47	.45	.43	.45	.43	.48	.50	.45
		.45	.45	.43	.45	.47	.47	.48	.43
	Average	.47	.46	.43	.45	.45	.48	.48	.44
	Maximum	.48	.47	.43	.45	.47	.52	.50	.45
	Minimum	.45	.45	.43	.45	.43	.45	.46	.43
	Max. difference	.03	.02	.00	.00	.04	.07	.05	.02
11125	Red	.45	.45	.42	.45	.47	.48	.47	.44
	Winter	.47	.45	.37	.43	.43	.43	.43	.44
		.48	.45	.43	.47	.47	.50	.50	.43
		...	...	...	.45	.45	.47	.47	.45
	Average	.47	.45	.41	.45	.45	.47	.47	.44
	Maximum	.48	.45	.43	.47	.47	.50	.50	.45
	Minimum	.45	.45	.37	.43	.43	.43	.43	.43
	Max. difference	.03	.00	.06	.04	.04	.07	.07	.02
11218	Common	.38	.40	.35	.37	.38	.38	.40	.38
	White	.37	.38	.37	.37	.37	.40	.42	.36
	(Winter)	.38	.37	.37	.38	.40	.40	.42	.36
		...	...	...	.37	.38	.42	.42	.37
	Average	.38	.38	.36	.37	.38	.40	.425	.37
	Maximum	.38	.40	.37	.38	.40	.42	.42	.38
	Minimum	.37	.37	.35	.37	.37	.38	.40	.36
	Max. difference	.01	.03	.02	.01	.03	.04	.03	.02
11251	Hard	.40	.38	.35	.35	.37	.38	.40	.38
	Red	.40	.38	.37	.37	.35	.42	.42	.38
	Winter	.40	.38	.37	.38	.38	.40	.42	.36
		.38	.38	.35	.37	.38	.45	.43	.38
	Average	.395	.38	.36	.37	.37	.41	.42	.375
	Maximum	.40	.38	.37	.38	.38	.42	.43	.38
	Minimum	.38	.38	.35	.35	.35	.38	.40	.36
	Max. difference	.02	.00	.02	.03	.03	.04	.03	.02
Av. max. difference		.024	.020	.026	.020	.036	.054	.044	.018
Av. ash % (5 samples)		.480	.471	.442	.455	.460	.476	.478	.451

*Ash completely fused.

As Series 2 indicated that fusion may lead to irregular results, tests were made fusing the ash at 685° C. and 785° C. The results show that fusion does cause irregular results, and also that, contrary to the general thought, fusion also resulted in higher test results.

The Hertwig and Bailey Method

The above tests all show need of improvement in the method if greater uniformity is to be the rule. Also the time—16 hours—is far too long for mill controlled work. In this connection, a method has been developed recently by Hertwig and Bailey who claim that by the use of a glycerol-alcohol mixture, and ash of desirable quality can be obtained in a very short time. To test the quality of the ash by this method, a fourth series of tests was made on the same five samples of flour, heating them at 570° C. for 16 hours. The results are given in column 8 of Table II. The ash was found to be in desirable form, white and fluffy. Moreover, the results did not vary greater than 0.02 per cent, with any one sample, the average difference being 0.018 per cent.

Knowing that the glycerol-alcohol mixture would produce a desirable quality of ash, the next step was to note whether, as claimed by the authors, the time could be shortened to meet average conditions.

To study the influence of the glycerol-alcohol mixture on the rapidity of ashing, two-, three-, and five-gram samples of flour were heated for varying lengths of time, as shown in Table III. Ashing for 16 hours at 575° C. is taken as correct.

The data show that a two-gram sample cannot be completely ashed in 2 hours. At three hours, a two-gram sample can be completely oxidized. An additional hour is necessary for a three-gram sample. The use of the glycerol-alcohol mixture, inasmuch as it produces a white to light gray ash, immediately eliminates the necessity of reheating and reweighing.

Size and Shape of Crucibles

Our experience has been in line with those recently reported by Hertwig and Bailey, namely, that combustion dishes of the Gooch crucible type, or with walls higher than 20 mm., retard the oxidation of flour ash. We do not find any advantage, however, in using silica dishes in preference to porcelain, or in using dishes of greater diameter than 40 mm. Dishes larger than this decrease the muffle capacity, increase the unit cost, and, if too large, influence the test, as they are difficult to weigh accurately.

TABLE III

EFFECT OF TIME AND SIZE OF SAMPLES ON ASH CONTENT OF SEVEN SAMPLES STRAIGHT WHEAT FLOUR

	Sample No. 11956	11966	11967	11974	11979	11982	12147	Av. max. difference	Av. ash
	%	%	%	%	%	%	%	%	%
Two-gram sample heated 2 hours, 575° C.									
	.44	.43	.49	.56	.43	.52	.51	....	...
	.40	.41	.49	.42	.41	.51	.50	....	...
	.40	.42	.49	.43	.45	.52	.53	....	...
Average	.41	.42	.49	.47	.43	.52	.51	....	.46
Max. dif.	.04	.02	.00	.14	.04	.01	.03	.026	...
Two-gram sample heated 2 hours, reheated 1 hour, 575° C.									
	.41	.40	.44	.45	.40	.47	.50	....	...
	.39	.41	.45	.44	.40	.47	.50	....	...
	.41	.41	.46	.44	.41	.47	.50	....	...
Average	.40	.41	.45	.44	.40	.47	.50	....	.44
Max. dif.	.02	.01	.02	.01	.01	.00	.00	.010	...
Two-gram sample heated 3 hours, 575° C.									
	.41	.46	.45	.45	.41	.48	.50	....	...
	.40	.40	.45	.44	.40	.48	.51	....	...
	.39	.40	.44	.44	.40	.48	.50	....	...
Average	.40	.40	.45	.44	.40	.48	.50	....	.44
Max. dif.	.02	.00	.01	.01	.01	.00	.01	.008	...
Three-gram sample heated 3 hours, 575° C.									
	.48	.43	.48	.63	.45	.49	.51	....	...
	.42	.41	.49	.48	.47	.51	.53	....	...
	.42	.42	.48	.49	.61	.49	.52	....	...
Average	.44	.42	.48	.53	.51	.50	.52	....	.49
Max. dif.	.06	.02	.01	.15	.16	.02	.02	.063	...
Three-gram sample heated 3 hours, reheated 1 hour, 575° C.									
	.41	.40	.46	.47	.40	.49	.50	....	...
	.41	.41	.46	.45	.42	.48	.50	....	...
	.41	.41	.46	.45	.42	.47	.50	....	...
Average	.41	.41	.46	.46	.41	.48	.50	....	.45
Max. dif.	.00	.01	.00	.02	.02	.02	.00	.010	...
Three-gram sample heated 4 hours, 575° C.									
	.42	.41	.45	.44	.39	.47	.50	....	...
	.40	.40	.44	.42	.41	.48	.49	....	...
	.40	.41	.45	.43	.41	.49	.51	....	...
Average	.41	.41	.45	.43	.40	.48	.50	....	.44
Max. dif.	.02	.01	.01	.02	.02	.02	.02	.016	...
Three-gram sample heated 17 hours, 575° C.									
	.41	.40	.44	.44	.40	.46	.50	....	...
	.41	.40	.44	.44	.40	.47	.51	....	...
	.40	.40	.44	.44	.40	.47	.50	....	...
Average	.41	.40	.44	.44	.40	.47	.50	....	.44
Max. dif.	.01	.00	.00	.00	.00	.01	.01	.004	...
Five-gram sample heated 17 hours, 575° C.									
	.40	.40	.44	.44	.40	.47	.50	....	...
	.40	.40	.44	.44	.40	.47	.50	....	...
	.40	.40	.45	.44	.40	.47	.50	....	...
Average	.40	.40	.44	.44	.40	.47	.50	....	.44
Max. dif.	.00	.00	.01	.00	.00	.00	.00	.002	...

Effect of Size of Sample on Ash Results

As different analysts use varying amounts of a sample for ash determinations, tests were made to determine the influence this factor has on ash results. One-, two-, three-, four-, and five-gram samples of high-ash and low-ash flours were ashed with the Hertwig-Bailey mixture in the same oven for a period of 16 hours. The results are given in Table IV.

TABLE IV
INFLUENCE OF SIZE OF SAMPLE ON THE ASH RESULTS

Sample No.	Grams of sample 1	2	3	4	5
	% Ash	% Ash	% Ash	% Ash	% Ash
12146	.36	.38	.36	.38	.38
"	.35	.37	.37	.38	.37
"	.36	.39	.38	.37	.38
"	.37	.38	.39	.39	.39
"	.38	.39	...	.38	.39
Av.	.364	.382	.375	.380	.382
Max. dif	.03	.02	.03	.02	.02
12127	.62	.60	.61	.59	.58
"	.59	.61	.60	.60	.59
"	.59	.59	.59	.60	.61
"	.57	.61	.59	.59	.60
"	.59	.61	...	.61	.58
Av.	.592	.604	.598	.598	.592
Max. dif	.05	.02	.02	.02	.03

The data show conclusively that identical results can be obtained with either a two-, three-, four-, or five-gram sample. A one-gram sample is too small for accurate weighing.

For the particular type of capsule used in these tests, a four- or five-gram sample makes the bulk rather deep for rapid work. Therefore, a two- or three-gram sample is recommended.

Conclusions

By the Hertwig-Bailey method, flour can be ashed in one heating in three hours.

To insure rapidity of oxidation, dishes not higher than 20 mm. should be used.

A dish 40 mm. in diameter will give satisfactory results.

A one-gram sample is too small for accurate work.

Fusion commences at 585° C., is complete with some samples at 600° C., and with all flour ash is fused at 685° C.

Fusion of flour ash causes high and irregular results.

BOOK REVIEWS

C. H. Bailey, The Chemistry of Wheat Flour, 324 pages, American Chemical Society Monograph Series, The Chemical Catalog Company, Inc., New York, 1925.

This monograph deals with the properties of flour in their relation to the raw material from which it is manufactured, or wheat; to the process of manufacture, or milling; and to the principal use to which the flour is to be put, or baking. It does not treat baking, except in so far as it is necessary to make clear the correlations between composition, or physico-chemical properties of flour, and baking qualities. As the author points out, baking in itself is a subject so important and so intricate it deserves treatment in a separate monograph. The book is, therefore, the only comprehensive treatise available upon the chemistry of wheat, including, as it does, with the more purely chemical subject matter, other material without which mere chemical data can neither be correlated nor understood. This amply justifies the inclusion of a discussion of the relationship of the different wheat races to one another, of the different varieties of wheat and their chemical composition and baking quality, of wheat breeding, of the growth and development of the wheat plant and kernel, and of the influence of environment on the composition of wheat. The book also includes a sound discussion of the defects and impurities in commercial wheat and of the storage and handling of wheat.

The chapter on the "Chemistry of Roller Milling" is really a chapter on milling unique in that it deals exhaustively and in a novel manner with the effect of the process of milling upon the chemical composition and baking quality of the various streams and separations. This is a field inadequately covered in the ordinary treatise on milling, though in the last few years, since flour is being bought more and more in America on chemical analysis, knowledge of the subject has become of vital importance in the operation of mills. The chapter is an excellent and original treatment of the subject.

The remainder of the book deals with the changes in flour incidental to aging, with the color of flour and flour bleaching, with flour strength and the colloidal behavior of dough. The treatment of these subjects is original and modern. The most recent literature is considered with sound critical judgment and perspective. The point of view is that of the physical and colloidal chemist. An equally clear, comprehensive and modern treatment is not to be found elsewhere—at least so far as the reviewer is aware.

The book is to be recommended unqualifiedly as a much needed and valuable contribution. It is, in the opinion of the reviewer, bound to

become the standard text on the subject. The reviewer has found no reasoning with which he is not disposed to agree and singularly few typographic errors. The only suggestion he has to make is that, inasmuch as the book covers so wide and diverse a field, in future editions a few of the more technical biological terms be explained, since they may be unfamiliar to some millers and mill chemists.

C. L. ALSBERG.

J. A. LeClerc, International Trade in Wheat and Wheat Flour, Trade Promotion Series No. 10, U. S Department of Commerce, Washington, 1925.

For the four years, 1920 to 1923 inclusive, wheat and flour exports from the United States averaged 22.5 per cent of all agricultural products shipped. The combined value of wheat and flour shipments in pre-war years averaged $101,000,000; in the 1920-1923 period, $466,000,000.

The present handbook attempts to give in detail the underlying basic facts with regard to the world's international trade in these commodities. It has been compiled from original sources. To facilitate comparisons, original units have been converted into bushels and barrels.

290 pages world production of wheat, by countries and grand divisions.

128 tables per capita acreage and production—wheat—grain shipments from surplus.

51 charts countries and imports into deficiency countries—wheat-flour exports—wheat-flour imports—grain trade by countries—consumption.

Price, 40 cents. Obtainable from the Superintendent of Documents, Government Printing Office, Washington, D. C.

INDEX—AUTHOR AND SUBJECT

EMPLOYMENT INFORMATION

The American Association of Cereal Chemists in convention at St. Louis, Mo., in June, 1925, voted to establish an employment service. A committee was appointed to administer this service of which Mr. C. B. Morison, 1135 Fullerton Avenue, Chicago, Ill., is chairman. Members of the association are extended the privilege of utilizing the services of this committee, and of publishing without charge suitable advertisements for Situations Wanted. CEREAL CHEMISTRY will publish these advertisements after they have been submitted to and approved by the Employment Committee. Correspondence and copies of advertisements should be sent to the Chairman of the Committee.

Non-members of the association may advertise in this journal for positions upon payment of one dollar per insertion of four lines plus twenty-five cents for each additional line.

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